Gel Electrophoresis of Proteins


At one time or another during the course of protein analysis or purification, researchers are likely to make use of gel electrophoresis. All laboratories working with proteins have some capability for carrying out gel electrophoresis and all researchers have at least rudimentary knowledge of the technique.

Gel electrophoresis can provide information about the molecular weights and charges of proteins, the subunit structures of proteins, and the purity of a particular protein preparation. It is relatively simple to use and it is highly reproducible. The most common use of gel electrophoresis is the qualitative analysis of complex mixtures of proteins. Microanalytical methods and sensitive, linear image analysis systems make gel electrophoresis popular for quantitative and preparative purposes as well. The technique provides the highest resolution of all methods available for separating proteins. Polypeptides differing in molecular weight by as little as a few hundreds of daltons and proteins differing by less than 0.1 pH unit in their isoelectric points are routinely resolved in gels.

Gel electrophoresis is a broad subject encompassing many different techniques. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is the most commonly practiced gel electrophoresis technique used for proteins. The method provides an easy way to estimate the number of polypeptides in a sample and thus assess the complexity of the sample or the purity of a preparation. SDS-PAGE is particularly useful for monitoring the fractions obtained during chromatographic or other purification procedures. It also allows samples from different sources to be compared for protein content. One of the more important features of SDS-PAGE is that it is a simple, reliable method with which to estimate the molecular weights of proteins. SDS-PAGE requires that proteins be denatured to their constituent polypeptide chains, so that it is limited in the information it can provide. In those situations where it is desirable to maintain biological activity or antigenicity, non-denaturing electrophoresis systems must be employed. However, the gel patterns from non-denaturing gels are more difficult to interpret than are those from SDS-PAGE. Non-denaturing systems also give information about the charge isomers of proteins, but this information is best obtained by isoelectric focusing (IEF; see the entry on IEF in the AES website). An IEF run will often show heterogeneity due to structural modifications that is not apparent in other types of electrophoresis. Proteins thought to be a single species by SDS-PAGE analysis are sometimes found by IEF to consist of multiple species. A true determination of the purity of a protein preparation is obtained with two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) that combines IEF with SDS-PAGE. Since 2-D PAGE is capable of resolving over 2,000 proteins in a single gel it is important as the primary tool of proteomics research where multiple proteins must be separated for parallel analysis (see the Application Focus on 2-D PAGE on this website). Proteins can be definitively identified by immunoblotting, which combines antibody specificity with the high resolution of gel electrophoresis (see the Application Focus on Immunoblotting on this website). Finally, gel electrophoresis lends itself to protein purification for which purpose
various devices have been developed (see the Application Focus on Preparative Electrophoresis on this website).

Although methods have been refined since the introduction of gel electrophoresis as an analytical technique, the basic principles and protocols have not changed appreciably. The topic has been covered in numerous, readily accessible texts, methods articles, and reviews (1-10).

The subject of electrophoresis deals with the controlled motion of charged particles in electric fields. Since proteins are charged molecules, they migrate under the influence of electric fields. From the point of view of electrophoresis, the two most important physical properties of proteins are their electrophoretic mobilities and their isoelectric points. The electrophoretic mobility of a protein depends on its charge, size, and shape, whereas its isoelectric point depends only on its net overall charge. Various electrophoresis systems have been developed to exploit the differences between proteins in these two fundamental properties.

The rate of migration of a protein per unit of field strength is called its “electrophoretic mobility”. The units of electrophoretic mobility are those of velocity (cm/sec) divided by the units of electric field (V/cm), or cm²/V-sec. Separations between proteins result from differences in their electrophoretic mobilities. It is relatively easy to show that in free solution the electrophoretic mobility of a particular protein is a function of the ratio of its charge to its frictional coefficient (shape) (1, 5, and 11). Both quantities are established by the composition of the protein and by the makeup of the surrounding medium. Electrophoretic mobilities are influenced by factors such as pH and the amounts and types of counter ions and denaturants that are present in the medium.

Proteins are amphoteric molecules. As such, they can carry positive, negative, or zero net charge depending on the pH of their local environment. For every protein there is a specific pH at which its net charge is zero. This pH is called the “isoelectric point”, or pI, of the protein. A protein is positively charged in solutions at pH values below its pI and negatively charged when the pH is above its pI. This pH dependence on charge obviously affects the mobilities of proteins in terms of both magnitude and the direction of migration. It is exploited in gel electrophoresis, especially in the technique of isoelectric focusing. (See the Application Focus on Isoelectric Focusing on this website.)

The electrophoretic mobilities of proteins are very different in gels than in free solution. Gels can act as molecular sieves for molecules the size of proteins. They consist of three-dimensional networks of solid material and pores. During electrophoresis in gels the polymeric material acts as a barrier to the motion of proteins forcing them to move between the buffer-filled pores of the gels.

Equipment and reagents for gel electrophoresis are readily available and familiar to laboratory workers. Particularly noteworthy is the steady increase in the popularity of precast polyacrylamide gels since their introduction in the early 1990s. Precast gels provide researchers with “off-the-shelf” convenience and help to make gel electrophoresis an “everyday” laboratory procedure.

1. **Gels**

   The key element in a gel electrophoresis system is, obviously, the gel itself. It determines the migration rates of proteins and holds proteins in place at the end of the run until they can be stained for visualization. Polyacrylamide gel is the principal medium for
protein electrophoresis. Agarose is used in some applications such as for the separation of proteins larger than about 500 kDa and for immunoelectrophoresis (6, 12). However, agarose gels are not used much in protein work and they are not discussed in this section.

Polyacrylamide gels are well suited for protein electrophoresis. (i) The gels can be cast in a range of pore sizes suitable for sieving proteins. (ii) The polymerization reaction is easy and reproducible and gels can be cast in a variety of shapes. (iii) Pore size is determined by the conditions of polymerization and can be easily altered by changing the monomer concentration. (iv) Polyacrylamide gels are hydrophilic and electrically neutral at the time they are cast. (v) They are transparent to light at wavelengths above about 250 nm and do not bind protein stains.

The gel-forming reaction is shown in *Figure 1*. Acrylamide and N,N’-methylenebisacrylamide (bis) are mixed, then copolymerized by means of a vinyl addition reaction initiated by free radicals. Gels are formed as acrylamide monomer polymerizes into long chains that are linked together by bis molecules. The resultant structure has both solid and liquid components. It can be thought of as a mass of relatively rigid fibers that create a network of spaces, all immersed in liquid. The liquid (buffer) in the gel maintains the gel’s three-dimensional shape. Without the liquid, the gel will dry to a thin film. At the same time, the polymer fibers prevent the liquid from flowing away.
Figure 1. (A) Polyacrylamide gel formation. Acrylamide and bis are copolymerized in a reaction catalyzed by ammonium persulfate and TEMED. (B) Hydrolysis of acrylamide to acrylate. Pendant neutral carboxamide groups can hydrolyze to charged carboxyls.

For protein electrophoresis, the pores of the gel are the important structures. During electrophoresis, proteins move through the pores of a gel. Nevertheless, the pore
size of a gel is difficult to measure directly. It is operationally defined by the size limit of proteins that can be forced through the gel.

From a macroscopic point of view, migrating proteins segregate into discrete regions, or zones, corresponding to their individual gel-mediated mobilities. When the electric field is turned off, the proteins stop moving. The gel matrix constrains the proteins at their final positions long enough for them to be stained to make them visible. An example of a one-dimensional separation of proteins is shown in Figure 2. In this configuration, the protein pattern is one of multiple bands with each band containing one protein or a limited number of proteins with similar molecular weights.

Figure 2. A typical analytical SDS-PAGE gel. Extracts of muscle proteins from five different fish varieties were separated by SDS-PAGE in a precast mid-size-gel and stained with colloidal CBB G-250 as described in the text. The lanes contain proteins from the following sources (left to right): two lanes of marker proteins, shark, salmon, trout, catfish, sturgeon, and rabbit (actin and myosin). The salmon and trout patterns (lanes 4 and 5) are very similar, as expected given the
close evolutionary relationship between the two species. All of the fish samples appear to contain muscle proteins similar to those of rabbit.

By convention, polyacrylamide gels are characterized by a pair of values, %T and %C (13). In this convention, %T is the weight percentage of total monomer (acrylamide + bis) in g/100 ml and %C is the proportion of bis as a percentage of total monomer. The effective pore size of a polyacrylamide gel is an inverse function of the total monomer concentration (%T) and a biphasic function of %C. When %T is increased at a fixed %C, the number of chains increases and the pore size decreases. On the other hand, when %T is held constant and %C is increased from low values, pore size decreases to a minimum at about 5% C. With further increases in %C from the minimum pore size increases, presumably because of the formation of shorter, thicker bundles of polymer chains. Gels with low %T (e.g., 7.5%T) are used to separate large proteins, while gels with high %T (e.g., 15%T) are used with small proteins.

An example of the effect of pore size on the separation of a set of native proteins is shown in Figure 3. The 4%T, 2.67%C gel is essentially non-sieving. Proteins migrate in it more-or-less on the basis of their free mobility. The 8%T, 2.67%C gel acts as a sieve for the proteins shown, demonstrating the combined effects of charge and size on protein separation. The relative positions of some proteins are shifted in the sieving gel as compared to the non-sieving one.
Figure 3. Effect of pore size on migration. The diagram shows the migration patterns of a set of proteins in the Ornstein-Davis, native, discontinuous system. The diagram on the left was obtained from 4%T, 2.67%C gels, while the pattern on the right was obtained from gels with 8%T, 2.67%C. The slanted lines connect bands representing the same proteins in the two gels. Note the large relative mobility shifts of BSA dimer and $\alpha$-lactalbumin between the two gels types.

1.1 Polymerization reactions

In the formation of polyacrylamide gels, the chemical system most often used to form the free radicals needed for polymerization consists of ammonium persulfate (APS) and N,N,N’,N’-tetramethylethylenediamine (TEMED). TEMED accelerates the decomposition of persulfate molecules into sulfate free radicals and these in turn initiate the polymerization. The free base of TEMED is required for this reaction, so that polymerization is most efficient at alkaline pH. Polymerization efficiency falls rapidly at pH values below about pH 6 (14). Photopolymerization with riboflavin and TEMED is used for low-pH gels (below pH 7).

The rate of polymerization is dependent on (i) the net concentration of monomers and initiators, (ii) the temperature, and (iii) the purity of the reagents. All three should be controlled for reproducibility. Reagents should be electrophoresis grade and water should be thoroughly deionized or distilled. For highest quality results, dissolved oxygen should be removed from the monomer mixtures by degassing them, since oxygen decreases the rate of polymerization (13). Nevertheless, completely acceptable gels can be obtained without removing oxygen, so that degassing can be omitted if it is inconvenient.
Monomers are commonly made as concentrated stock solutions containing 30\%T or 40\%T in water. For proteins, the usual crosslinker concentration is 2.7\%C. These stock solutions can be purchased commercially. Other gel-casting reagents include buffers and initiators. Monomer stock and buffer are combined at the desired concentrations and deaerated under moderate vacuum for about 15 min. Initiators are then added and the solution is poured into the casting apparatus.

Initiator concentrations are determined empirically to give visible polymerization in 15-20 min after addition. Under these conditions, gelation is essentially complete in 90 min (15). Final ammonium persulfate and TEMED concentrations of 0.05\% each are usually sufficient for polymerization of resolving gels (see Section 2.2.). When stacking gels are used (see Section 2.2.), all that is required of them is that they have large pores. Because of this, stacking gels can be set to polymerize rapidly, in 8-10 min, with final concentrations of 0.05\% ammonium persulfate and 0.1\% TEMED.

Polyacrylamide gels are inherently unstable (16). At basic pH, the pendant neutral carboxamide groups (-CO-NH$_2$) of acrylamide monomers hydrolyze to ionized carboxyl groups (-COO$^-$) which can interact with some proteins (Figure 1). In addition, counter ions from the buffer neutralize the carboxyl groups. The waters of hydration associated with the counter ions disrupt the integrity of the pores. Over extended periods of storage, band sharpness and resolution slowly deteriorate. This becomes noticeable after storage at 4°C for 3 to 4 months depending on the gel type and the buffer. The aging of polyacrylamide gels has commercial significance because it limits the shelf lives of precast gels (see below).

2. Buffers

The electrical current in an electrophoresis cell is carried largely by the ions supplied by buffer compounds - proteins constitute only a small portion of the current carrying ions in an electrophoresis cell. Buffers supply current carrying ions, maintain desired pH, and provide a medium for heat dissipation. In native systems, electrophoresis buffers also maintain the pH environment needed for protein activity.

Many useful buffer systems have been devised, but only a few are in widespread use (1-7, 13). Buffer systems for electrophoresis are classified as either continuous or discontinuous, depending on whether one or more buffers are used. Both types of buffer system are useful. Dilute samples require discontinuous systems for best results. With high-concentration protein samples, above about 1 mg/ml, continuous buffer systems provide adequate resolution (17).

2.1 Continuous buffer systems

Continuous systems use the same buffer, at constant pH, in the gel, sample, and electrode reservoirs. With continuous systems, the sample is loaded directly on the gel in which separation will occur. It is helpful to dilute the sample buffer to at least half strength. The decrease in conductivity achieved by dilution causes a localized voltage drop across the sample that helps drive proteins into the gel. As proteins migrate through the pores of the gel they are separated on the basis of (gel-mediated) mobility differences. Bandwidths are highly dependent on the height of the applied sample volume, which should be kept as small as possible, thus restricting continuous systems to high-concentration samples for best results.
Almost any buffer can be used for continuous-buffer electrophoresis. Solutions of relatively low ionic strength are best suited for electrophoresis, because these keep heat generation at a minimum. On the other hand, protein aggregation may occur if the ionic strength is too low. The choice of buffer will depend on the proteins being studied, but in general, the concentrations of electrophoresis buffers are in the range of from 0.01 to 0.1 M (but see Protocol 3).

2.2 Discontinuous buffer systems

Discontinuous buffer systems (often called multiphasic buffer systems) employ different ions in the gel and electrode solutions. These systems are designed to sharpen starting zones for high-resolution separations, even with dilute samples. The sharpening of sample starting zones is called “stacking”. It is an electrochemical phenomenon based on mobility differences between proteins and carefully chosen leading and trailing buffer ions. Samples are diluted in gel buffer and sandwiched between the gel and the electrode buffer. When the electric field is applied, leading ions from the gel move ahead of the sample proteins while trailing ions from the electrode buffer migrate behind the proteins. The proteins in the sample become aligned between the leading and trailing ion fronts in the order of decreasing mobility. Proteins are said to be stacked between the two buffer ion fronts. The width of the stack is no more than a few hundred micrometers with protein concentrations there approaching 100 mg/ml (18). Electrophoretic stacking concentrates proteins into regions narrower than can be achieved by mechanical means. This has the effect of minimizing overall bandwidths during a run.

In order to allow the stack to develop, the gels used with discontinuous systems are usually divided into two distinct segments. The smaller, upper portion is called the stacking gel. It is cast with appreciably larger pores than the lower resolving gel (or separating gel) and serves mainly as an anticonvective medium during the stacking process. Separation takes place in the resolving gel, which has pores of roughly the same size as the proteins of interest. Once proteins enter the resolving gel their migration rates are slowed by the sieving effect of the small pores. In the resolving gel, the trailing ions pass the proteins and electrophoresis continues in the environment supplied by the electrode buffer. The proteins are said to become “unstacked” in the resolving gel. They separate there on the basis of size and charge.

Runs are monitored and timed by means of the buffer front. The migration of the buffer front as it moves through the gel can be followed by the change in the index of refraction between the regions containing the leading and trailing ions. Tracking dye that moves with the buffer front aids in visualization of its motion.

For detailed descriptions of the electrochemical processes that operate with discontinuous buffer systems, consult refs. 1-7, 13, and 19. Mathematically inclined readers might want to follow the development of multiphasic buffer theory as presented in References 20-22.

2.3 Native systems

The choice of electrophoresis system depends on the particular proteins of interest. There is no universal buffer system ideal for the electrophoresis of all native proteins. Both protein stability and resolution are important considerations in buffer
selection. Recommend choices are the Ornstein-Davis discontinuous system (20, 23) and McLellan’s continuous buffers (24).

The set of buffers compiled by McLellan provide the simplest way to carry out the electrophoresis of proteins in their native state (24). McLellan’s buffers range from pH 3.8 to pH 10.2, all with relatively low conductivity (Table 1). By using different buffers from the set it is possible to compare the effect of pH changes on protein mobility while maintaining similar electrical conditions. This is demonstrated in Figure 4. The illustration is a line-drawing representation, drawn to scale, of the relative positions of two hemoglobin variants, A and C, run under comparable electrical conditions in different McLellan buffers. HbA has a lower isoelectric point (pI 7.1) than HbC (pI 7.4). At pH 7.4 neither protein carries enough charge to move into the gel. At the acidic pHs tested, HbC is more highly charged and moves further through the gel than HbA. The situation is reversed at the basic pHs tested. Note the differences in polarity and run times of the various runs.

<table>
<thead>
<tr>
<th>pH</th>
<th>Buffer</th>
<th>Basic component</th>
<th>Amount for 5X solution</th>
<th>Acidic component</th>
<th>Amount for 5X solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.8</td>
<td>β-Alanine</td>
<td>1X = 30 mM</td>
<td>13.36 g/liter</td>
<td>Lactic acid</td>
<td>1X = 20 mM</td>
</tr>
<tr>
<td>4.4</td>
<td>β-Alanine</td>
<td>1X = 80 mM</td>
<td>35.64 g/liter</td>
<td>Acetic acid</td>
<td>1X = 40 mM</td>
</tr>
<tr>
<td>4.8</td>
<td>Gaba</td>
<td>1X = 80 mM</td>
<td>41.24 g/liter</td>
<td>Acetic acid</td>
<td>1X = 20 mM</td>
</tr>
<tr>
<td>6.1</td>
<td>Histidine</td>
<td>1X = 30 mM</td>
<td>23.28 g/liter</td>
<td>Mes</td>
<td>1X = 30 mM</td>
</tr>
<tr>
<td>6.6</td>
<td>Histidine</td>
<td>1X = 25 mM</td>
<td>19.4 g/liter</td>
<td>Mops</td>
<td>1X = 30 mM</td>
</tr>
<tr>
<td>7.4</td>
<td>Imidazole</td>
<td>1X = 43 mM</td>
<td>14.64 g/liter</td>
<td>Hepes</td>
<td>1X = 35 mM</td>
</tr>
<tr>
<td>8.1</td>
<td>Tris</td>
<td>1X = 32 mM</td>
<td>19.38 g/liter</td>
<td>Epps</td>
<td>1X = 30 mM</td>
</tr>
<tr>
<td>8.7</td>
<td>Tris</td>
<td>1X = 50 mM</td>
<td>30.29 g/liter</td>
<td>Boric acid</td>
<td>1X = 25 mM</td>
</tr>
<tr>
<td>9.4</td>
<td>Tris</td>
<td>1X = 60 mM</td>
<td>36.34 g/liter</td>
<td>Caps</td>
<td>1X = 40 mM</td>
</tr>
</tbody>
</table>
10.2 Ammonia 12.5 ml/liter Caps 22.13 g/liter
1X = 37 mM 1X = 20 mM

bListed buffer pH is ±0.1 unit. Do not adjust the pH with acid or base. Remake buffers outside the given range.
cLactic acid from an 85% solution.

g

Figure 4. Effect of pH on mobility. Hemoglobin A (pI 7.1) and Hemoglobin C (pI 7.4) were run in native, continuous systems using eight of the McLellan buffers (Table 1). Bands marked “A” and “C” show the positions of the two hemoglobin variants in each gel representation. The polarity of the voltages applied to the electrophoresis cell is indicated by + and – signs above and below the vertical arrows. Note the polarity change between the gel at pH 7.4 and the one at pH 8.2. This reflects the pIs of the two proteins (and was accomplished by reversing the leads of the electrophoresis cell at the power supply).

Protocol 1. Casting gels for the McLellan native, continuous buffer systems

Equipment and reagents
- Gel cassettes and casting apparatus for the electrophoresis cell being used.
- Acrylamide concentrate (30%T, 2.7%C)b
- 5X gel buffer. See Table 1.
- 10% ammonium persulfate (APS)c
- N,N,N',N'-tetramethylethylene diamine (TEMED)d
Method

This method is for casting two mini gels.

1. Prepare the desired 5X buffer. See Table 1.

2. Thoroughly clean the glass plates, spacers, and combs with detergent and rinse them well with water.

3. Assemble the cassettes and place them in the casting apparatus.

4. Determine the gel volume from the manufacturer’s specifications, by calculation, or by measuring the amount of water needed to fill a cassette.

5. Insert the well-forming combs between the gel plates of the cassettes and tilt them at a slight angle to provide a way for air bubbles to escape.

6. Mix

   12 ml of water
   4 ml of 5X buffer
   4 ml of stock 30%T, 2.7%C acrylamide solution
   100 µl of 10% APS
   20 µl of TEMED

   Swirl the solution gently and transfer it to the cassettes using a pipette and bulb.

7. Align the comb to its proper position being careful to not trap bubbles under the teeth. The gel should be ready to use in about 90 minutes.

   Note: The APS-TEMED pair is a less efficient polymerization initiator below pH 6 than it is above pH 6. To compensate for this loss of efficiency in buffers below pH 6, increase the concentration of TEMED five-fold. Alternatively, add 100 µg of riboflavin 5’-monophosphate (100 µl of a 0.1% solution) to the solution prepared as in Step 6 above and initiate photopolymerization as is done with IEF gels (see the Application Focus about Isoelectric Focusing on this web site).

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See Ref. 24.

To prepare 30%T, 2.7%C acrylamide stock solution, dissolve 29.2 g of acrylamide and 0.8 g of bisacrylamide in 72.5 ml of water. The final volume will be 100 ml. Both this solution and premixed acrylamide-bisacrylamide powder are commercially available.

To prepare 10% APS, dissolve 100 mg of APS in 1 ml of water. APS solutions should be made fresh daily, but may perform satisfactorily for up to a week. Store at room temperature.

TEMED is used undiluted as supplied.

Caution: Acrylamide monomer is toxic. Avoid breathing acrylamide dust, do not pipette acrylamide solutions by mouth, and wear gloves when handling acrylamide powder or solutions containing it. Allow unused monomer solutions to polymerize and discard the resultant gels.

Other buffers that have been used for continuous, native electrophoresis are Tris-glycine (pH range 8.3-9.5) (17), Tris-borate (pH range 8.3-9.3) (25), and Tris-acetate (pH
range 7.2-8.5) (26). Borate ions can form complexes with some sugars and can therefore influence resolution of some glycoproteins. Very basic proteins, such as histones and ribosomal proteins, are separated in acetic acid-urea gels (27). These buffer systems are not presented in protocols here in the interest of space.

Ornstein (20) and Davis (23) developed the first high-resolution PAGE system for native (non-denatured) proteins. Their popular system is still in widespread use. It was designed for the analysis of serum proteins, but works well for a broad range of protein types. The Ornstein-Davis buffers should be the first discontinuous system tried when working with a new, native sample.

Gels for the Ornstein-Davis method are cast in two sections. A large-pore stacking gel (4%T, 2.7%C) is cast on top of a small-pore resolving gel (from 5 to 30%T depending on the proteins being studied). The two gel sections also contain different buffers. The stacking gel contains 0.125 M Tris-Cl, pH 6.8, and the resolving gel contains 0.375 M Tris-Cl, pH 8.8. Sample is diluted in 0.0625 M Tris-Cl, pH 6.8. The electrode (or running) buffer is 0.025 M Tris, 0.192 M glycine, pH 8.3. The pH discontinuity between the two sections of the gel was designed to regulate the effective mobility of glycinate ions from the cathode chamber. The concentrations of all four buffers were derived from electrochemical considerations based on the properties of serum proteins. The porosity of the resolving gel must be empirically determined to match the mobilities of the proteins in the sample. There is no reliable way to predict the correct gel concentration of an untested protein mixture without analyzing the proteins in gels. The choice is made such that the proteins of interest in the sample mixture are resolved in the gel. It is common to begin with a 7.5%T gel for the initial electrophoresis of a sample of unknown mobilities, then to try higher concentration gels (and sometimes lower concentration gels, such as 5%T).

For basic proteins, the low-pH alanine-acetate system of Reisfeld et al. (28) is often used. Allen and Budowle suggest Tris-sulfate/Tris-borate, Tris-formate/Tris-borate, and Tris-citrate-Tris-borate (4) (not presented as protocols).

### 2.4 Denaturing systems

Because it is not yet possible to calculate the physical properties of proteins from mobility data, researchers have taken to denaturing SDS-PAGE in order to estimate protein molecular weights. Sample treatment for SDS-PAGE breaks all inter- and intramolecular bonds, both covalent and non-covalent, and leaves the polypeptide subunits of proteins in forms that can be separated on the basis of their molecular weights. Moreover, SDS solubilizes most proteins, so SDS-PAGE is applicable to a wide range of sample types. The electrophoretic band patterns obtained by SDS-PAGE are appreciably easier to interpret than those from native PAGE.

The most popular electrophoresis system is the discontinuous buffer system devised by Laemmli (29). Laemmli added SDS to the standard Ornstein-Davis buffers (Tables 2 and 3) and developed a simple denaturing treatment.
Table 2. Compositions of electrode buffers

McLellan native, continuous buffer systems

5X buffer stocks

See Table 1.

Store at room temperature. To prepare 1X electrode buffer, dilute 200 ml of 5X buffer stock with 800 ml of water (see Table 1).

Ornstein-Davis native, discontinuous buffer

10X electrode buffer stock

| Tris base | 30.3 g |
| Glycine   | 144.0 g |

Dissolve in water to a total volume of 1 liter. Do not adjust the pH. Store at room temperature. To prepare 1X electrode buffer (0.025 M Tris, 0.192 M glycine, pH 8.3), dilute 100 ml of 10X buffer stock with 900 ml of water.

Laemmli SDS-PAGE buffer

10X electrode buffer stock

| Tris base   | 30.3 g |
| Glycine     | 144.0 g |
| SDS         | 10.0 g |

Dissolve in water to a total volume of 1 liter. Do not adjust the pH. Store at room temperature. To prepare 1X electrode buffer (0.025 M Tris, 0.192 M glycine, 0.1% SDS, pH 8.3), dilute 100 ml of 10X buffer stock with 900 ml of water.

Tricine SDS-PAGE buffer

10X electrode buffer stock

| Tris base  | 12.1 g |
| Tricine    | 17.9 g |
| SDS        | 10.0 g |

Dissolve in water to a total volume of 1 liter. Do not adjust the pH. Store at room temperature. To prepare 1X electrode buffer (0.1 M Tris, 0.1 M Tricine, 0.1% SDS, pH 8.3), dilute 100 ml of 10X buffer stock with 900 ml of water.

Table 3. Compositions of sample buffers

McLellan native, continuous buffer systems
0.5X gel buffer, 10% (w/v) glycerol, 0.01% bromophenol blue

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X Gel buffer</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>50% Glycerol</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>0.5% Bromophenol blue</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Water</td>
<td>6.8 ml</td>
</tr>
</tbody>
</table>

Store at room temperature.

**Ornstein-Davis native, discontinuous buffer**

0.0625 M Tris-Cl, pH 6.8, 10% glycerol, 0.01% bromophenol blue

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stacking gel 4X buffer stock</td>
<td>1.25 ml</td>
</tr>
<tr>
<td>50% (w/v) Glycerol</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>0.5% Bromophenol blue</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Water</td>
<td>6.55 ml</td>
</tr>
</tbody>
</table>

Store at room temperature.

**Laemmli SDS-PAGE buffer**

0.075 M Tris-Cl, pH 8.8, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.01% bromophenol blue

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock sample buffer</td>
<td></td>
</tr>
<tr>
<td>Resolving gel 4X buffer stock</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>10% SDS</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>50% (w/v) Glycerol</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>0.5% Bromophenol blue</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Water</td>
<td>4.8 ml</td>
</tr>
</tbody>
</table>

Store at room temperature. Prior to use, add 50 µl of 2-mercaptoethanol to 950 µl of sample buffer stock. An alternative sample buffer that is often used is obtained by adjusting the Ornstein-Davis sample buffer to 2% SDS and 5% 2-mercaptoethanol.

**Tricine SDS-PAGE sample buffer**

0.1 M Tris-Cl, pH 8.45, 1% SDS, 2% 2-mercaptoethanol, 20% glycerol, 0.04% CBB G-250

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock sample buffer</td>
<td></td>
</tr>
<tr>
<td>Tricine gel 3X buffer stock</td>
<td>0.33 ml</td>
</tr>
<tr>
<td>10% SDS</td>
<td>1 ml</td>
</tr>
<tr>
<td>50% (w/v) Glycerol</td>
<td>4 ml</td>
</tr>
<tr>
<td>0.5% CBB G-250</td>
<td>0.4 ml</td>
</tr>
<tr>
<td>Water</td>
<td>3.77 ml</td>
</tr>
</tbody>
</table>

Store at room temperature. Prior to use, add 20 µl of 2-mercaptoethanol to 980 µl of buffer.

---

*5X McLellan gel buffer. See Table 1.
*b50% (w/v) Glycerol. See Protocol 3.
*c0.5% Bromophenol blue. Dissolve 50 mg of bromophenol blue in 10 ml of water.
*dStacking gel 4X buffer stock. See Protocol 2.
*eResolving gel 4X buffer stock. See Protocol 2.
*f10% SDS. Dissolve 10 g of SDS in ~70 ml of water, then adjust the volume to 100 ml.
*gTricine gel 3X buffer stock. See Protocol 3.
*h0.5% Coomassie Brilliant Blue G-250. Dissolve 50 mg of CBB G-250 in 10 ml of water.
Sample preparation for SDS-PAGE is quite easy. Proteins are simply brought to near boiling in dilute gel buffer containing 5% (v/v) 2-mercaptoethanol, a thiol reducing agent, and 2% (w/v) SDS. The treatment simultaneously breaks disulfide bonds and dissociates proteins into their constituent polypeptide subunits. SDS monomer binds to the polypeptides and causes a change in their conformations. For most proteins, 1.4 g of SDS binds per gram of polypeptide (approximately one SDS molecule per two amino acids) (30). The properties of the detergent overwhelm the properties of the polypeptides. In particular, the charge densities of SDS-polypeptides are independent of pH in the range from 7 to 10 (4, 31). Most significantly, the SDS-polypeptides all assume the same hydrodynamic shape (30, 32). This means that their electrophoretic mobilities are nearly identical. If electrophoresis were done on a mixture of SDS-polypeptides in free solution, they would all migrate together. In gels, SDS-polypeptides separate by sieving on the basis of size (molecular weight of the polypeptide). Laemmli’s buffers, as usually described, are more elaborate than strictly necessary. Most presentations of this method utilize the two different gel buffers of the Ornstein-Davis system with SDS added to them. Because SDS so dominates the electrophoresis system, the buffer in the stacking gel can be the same as the buffer in the resolving gel. Results are the same whether the stacking gel is cast at pH 6.8 or at pH 8.8. Also, gels do not need to be cast with SDS in them (31). The SDS in the sample buffer is sufficient to saturate the proteins with the detergent. SDS in the cathode buffer overtakes the proteins in the sample and at 0.1% is sufficient for maintaining protein saturation during electrophoresis. This distinction is important for the commercial manufacturing of gels for SDS-PAGE (see Section 10.).

**Protocol 2. Casting gels for the Ornstein-Davis native buffer system**\(^a\) and the Laemmli SDS-PAGE system\(^b\)

**Equipment and reagents**
- Gel cassettes and casting apparatus
- Acrylamide concentrated stock solution (30%T, 2.7%C). See Protocol 1.
- APS and TEMED. See Protocol 1.
- 2-butanol (water saturated)\(^c\)
- Resolving-gel 4X buffer stock\(^d\)
- Stacking-gel 4X buffer stock
  - For native PAGE\(^e\)
  - For SDS-PAGE\(^f\)

**Method**

This method is for preparing two mini gels.

1. Thoroughly clean the glass plates, spacers, and combs with detergent and rinse them well with water. Assemble the casting apparatus according to the manufacturer’s instructions.

2. Mark the glass at the height of the resolving gels, which should be 0.5-1 cm below the bottoms of the wells (comb teeth).

3. Calculate the amount of 30%T acrylamide stock solution is needed to make 20 ml of monomer solution at the desired %T for the resolving gel. 
   \[V_{\text{resolver}} = \frac{2}{3}(\%T_{\text{resolver}}), \text{ in ml.}\]
4. Calculate the amount of water to be used for the resolving gel. 
   \[ V_{\text{water}} = (15 - V_{\text{resolver}}) \text{, in ml.} \]

5. Combine the components of the resolving gel monomer mixture:
   \[ \begin{align*}
   \text{30\% T acrylamide stock solution} & : V_{\text{resolver}} \text{ ml} \\
   \text{water} & : V_{\text{water}} \text{ ml} \\
   \text{resolving-gel 4X stock solution} & : 5 \text{ ml} \\
   \text{10\% APS} & : 100 \mu\text{l} \\
   \text{TEMED} & : 10 \mu\text{l}.
   \end{align*} \]
   Swirl the solution gently and transfer it immediately to the gel cassettes using a pipette and bulb. Add the monomer mixture only to the mark showing gel height.

6. Overlay the resolving gel solution with water-saturated 2-butanol to exclude air from the top of the gel. Allow the gel to polymerize for about 1 hour. Polymerization is evidenced by the appearance of a sharp interface beneath the overlay, which should start to become evident in about 15 min. Polymerization is essentially complete in about 90 min.

7. Once the resolving gels have hardened, rinse the tops of them thoroughly with water and dry the areas above them with filter paper. Place well-forming combs between the plates and tilt them at a slight angle to provide a way for bubbles to escape.

8. Combine the components of the stacking gel monomer mixture (10 ml):
   \[ \begin{align*}
   \text{Water} & : 6.2 \text{ ml} \\
   \text{30\% T acrylamide stock solution} & : 1.3 \text{ ml} \\
   \text{stacking-gel 4X stock solution} & : 2.5 \text{ ml} \\
   \text{10\% APS} & : 50 \mu\text{l} \\
   \text{TEMED} & : 10 \mu\text{l}.
   \end{align*} \]
   Swirl the solution gently and transfer it to the cassettes on top of the resolving gels using a pipette and bulb. Align the comb to its proper position being careful to not trap bubbles under the teeth. No overlay is required because the comb excludes air from the wells. Allow the gel to polymerize for about an hour.

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*See refs. 20 and 23.
*See ref. 29.

To prepare 2-butanol, saturate a quantity (≤100 ml) of 2-butanol with water.

To prepare resolving-gel 4X stock (1.5 M Tris-Cl, pH 8.8), dissolve 18.2 g of Tris base in ~80 ml of water, adjust the solution to pH 8.8 with HCl and add water to a final volume of 100 ml. Store this solution at room temperature. This solution is commercially available.

To prepare 4X stacking-gel buffer stock for native PAGE (0.5 M Tris-Cl, pH 6.8) dissolve 6.1 g of Tris base in ~80 ml of water, adjust the pH of the solution to pH 6.8 and add water to 100 ml. Store this solution at room temperature. This solution is commercially available.

4X stacking-gel buffer stock for SDS-PAGE is the same as resolving-gel 4X stock buffer.

Caution. Acrylamide monomer is toxic. Avoid breathing acrylamide dust, do not pipette acrylamide solutions by mouth, and wear gloves when handling acrylamide powder or solutions containing it. Allow unused acrylamide monomer solutions to polymerize and discard the resultant gels.
Other systems for SDS-PAGE have been developed (not presented in protocols). Weber and Osborn’s continuous, denaturing SDS-PAGE system uses pH 7 sodium phosphate buffer (33). This system helped establish the utility of SDS in electrophoresis as a means for estimating the molecular weights of proteins. The Weber-Osborn system is a popular one, but the lack of stacking limits its use to high-concentration samples for best resolution. A protocol for the Weber-Osborn system can be found in ref. 34.

Neville (35) adapted a Tris-sulfate/Tris borate buffer system to fractionate SDS-saturated proteins in the 2 to 300 kDa range. The Neville system produces very sharp bands. Wykoff et al. (31) replaced Tris in the Laemmli SDS-PAGE system with its analog ammediol (2-amino-2-methyl-1,3-propanediol). The ammediol system resolves better in the 1 to 10 kDa size range than either the Laemmli or Neville systems, but the bands are less sharp.

The cationic detergent cetyltrimethylammonium bromide (CTAB) has been used as an alternative to SDS for gel electrophoresis of proteins. Akins et al. (36) devised the most successful application of CTAB. The Akins method employs a discontinuous buffer system with sodium (from NaOH) as the leading ion and arginine as the trailing ion with Tricine as the counter ion and buffer. This method uses no reducing agent in the sample buffer and protein solutions are not boiled prior to electrophoresis. As a result, many enzymes retain their activities. Nonetheless, CTAB coats proteins thoroughly enough that it can be used for molecular weight determinations in analogy with SDS-peptides. See ref. 34 for a protocol for CTAB-PAGE.

A system based on the use of Tricine instead of glycine in the electrode buffer provides excellent separation of small polypeptides (37). Peptides as small as 1 kDa are resolvable in Tricine-SDS gels. In particular, 16.5%T, 3%C separating gels are used for separations in the range from 1 to 70 kDa. Stacking gels in this system are 4%T, 3%C. Resolution is sometimes enhanced by inclusion of a 10%T, 3%C spacer gel between the resolving and stacking gels. Tricine-SDS resolving gels contain 1 M Tris-Cl, pH 8.45, and 13% glycerol. It is not necessary to include SDS in the gel buffer, but the glycerol is important to impart a viscosity that seems necessary for resolving small peptides. Electrode buffer is 0.1 M Tris, 0.1 M Tricine, 0.1% SDS, pH 8.25. Sample buffer is 0.1 M Tris-Cl, pH 8.45, 1% (w/v) SDS, 2% (v/v) 2-mercaptoethanol, 20% (w/v) glycerol, 0.04% Coomassie Brilliant Blue G-250. Sample buffer should contain no more than 1% SDS for best resolution of small polypeptides (1-5 kDa). Proteins of very low molecular mass are not completely fixed and may diffuse from the gels during staining. This system is quite popular for polypeptide analysis (Protocol 3).

**Protocol 3. Casting gels for Tricine SDS-PAGE**

*Equipment and reagents*

- 40%T, 3%C acrylamide stock solution
- Gel buffer 3X stock solution
- All other equipment and reagents are the same as for Protocol 2.

*Method*

This method is for casting two 16.5%T mini gels.
1. Assemble the gel cassettes and casting apparatus as in Protocol 2.

2. Combine the components of the resolving gels (20 ml):
   
<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>3.0 ml</td>
</tr>
<tr>
<td>40% T acrylamide stock solution</td>
<td>8.2 ml</td>
</tr>
<tr>
<td>Gel buffer 3X stock solution</td>
<td>6.7 ml</td>
</tr>
<tr>
<td>Glycerol</td>
<td>2.6 g</td>
</tr>
<tr>
<td>10% APS</td>
<td>100 µl</td>
</tr>
<tr>
<td>TEMED</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Mix the components until all the components dissolve and transfer the solution to the gel cassettes using a pipette and bulb. Leave space in the cassettes for the stacking gels. Allow the gels to polymerize for about 1 hour.

3. Prepare the cassettes for the stacking gels by rinsing the tops of the resolving gels with water, drying the spaces above the resolving gels with filter paper, and inserting combs (at a slight angle).

4. Combine the components of the stacking gels (10 ml):
   
<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>5.7 ml</td>
</tr>
<tr>
<td>40% T acrylamide stock solution</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Gel buffer 3X stock solution</td>
<td>3.3 ml</td>
</tr>
<tr>
<td>10% APS</td>
<td>50 µl</td>
</tr>
<tr>
<td>TEMED</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Swirl the solution gently to mix it and transfer it to the cassettes using a pipette and bulb.

5. Allow the gels to polymerize for about an hour before using them.

\(^a\)See ref. 37.

\(^b\)To prepare 40% T, 3% C acrylamide solution, dissolve 38.8 g of acrylamide and 1.2 g of bisacrylamide in 63.6 ml of water. The final volume will be 100 ml. This solution is commercially available.

\(^c\)To prepare 3X gel buffer (3 M Tris-Cl, pH 8.45), dissolve 36.3 g Tris base in ~80 ml of water, adjust the solution to pH 8.45 with HCl, and add water to a final volume of 100 ml. Store the solution at room temperature.

See the Caution note in Protocol 2.

Compositions of electrode and sample buffers can be found in Tables 2 and 3, respectively.

3. **Pore-size gradient gels**

Acrylamide allows for the possibility of casting pore-gradient gels. Pore-gradient gels are used in SDS-PAGE applications where they have some advantages over homogeneous gels. The most common gradient gels are usually cast with acrylamide concentrations that increase linearly from top to bottom so that the pores get smaller with the distance into the gels. As proteins move through gradient gels from regions of relatively large pores to regions of relatively small pores, their migration rates slow down. Small proteins remain in gradient gels much longer than they do in single-percentage gels, so that they allow both large and small molecules to be run in the same gel. This makes gradient gels popular for analyses of complex mixtures spanning wide
molecular mass ranges. A gradient gel, however, cannot match the resolution obtainable with a properly chosen single concentration of acrylamide. A good approach is to use gradient gels for estimates of the complexities of mixtures. This may be sufficient for some purposes. However, best resolution requires the appropriate single-concentration gel.

Various devices are commercially available for producing polyacrylamide gradients of almost any desired shape. These devices range in complexity from programmable pumps to simple cylinder pairs that form gradients hydrostatically. It is much simpler to purchase commercially available precast gradient gels (Section 10.). A protocol for casting gradient gels is available in Reference 34.

Discontinuous buffer systems give best resolution in gradient gels: 4-15%T gradient mini-gels, based on the Laemmli buffers, resolve SDS-polypeptides in the 40 to 200-kDa size range, 4-20%T gels separate 10 to 100-kDa SDS-proteins, and 10-20%T gradients are useful in the 10-100-kDa range.

4. Apparatus

Apparatus for gel electrophoresis are relatively simple (Figure 5). Electrophoresis cells are essentially plastic boxes with anode and cathode buffer compartments, electrodes (usually platinum wire), and jacks for making electrical contact with the electrodes. Gels are held vertically between the electrode chambers during the run. Gel cassettes have open tops and bottoms. The bottom is sealed with a gasket during gel formation and the top is open to receive monomer solution. The top and bottom ends are open and in contact with buffer for electrophoresis. High-voltage direct current supplies provide electrical power for electrophoresis. Micropipettes, test tubes, and heating blocks are sample-handling necessities. Many suitable devices are available from a number of suppliers.
Figure 5. Exploded view of an electrophoresis cell. The components of the Bio-Rad Mini-PROTEAN 3 cell are shown. The inner chamber assembly can hold two gels. It contains an electrode assembly and a clamping frame. The interior of the inner assembly constitutes the upper buffer compartment (usually the cathode). The chamber is placed in the tank, which is filled with buffer and constitutes the lower (anode) buffer compartment. Electrical contact is made through the lid.
Gels are cast as rectangular slabs in glass or plastic cassettes. The slab format provides uniformity, so that different samples can be compared in the same gel. In a small number of applications, gels are cast in cylindrical glass tubes. For comparative purposes, slab gels are far superior to tube gels. Conventional gels are of the order of 20 cm long and 20 cm wide. Gel thickness is varied by means of spacers inserted into the cassettes prior to gel formation. A thickness of 0.75- or 1-mm allows for adequate loads for good sensitivity of detection while at the same time allowing relatively high voltages to be applied to the gel without excessive heating. Runs take from 2.5 to 6 hr to complete.

Some cells provide means for cooling, but this is not usually necessary. Cooling is often required to maintain the activity of proteins in the native state. It is not necessary with denaturing conditions in which protein conformations are intentionally destroyed. In some cases, cooling is undesirable. SDS will begin to crystallize out of solution at temperatures below about 10°C, so excessive cooling should be avoided in SDS-PAGE. When cooling is distributed unevenly across a gel cassette the apparent bandwidths can be distorted. For example, with some cell designs, cooling is applied to only one face of the gel cassette. Proteins near the cooled face of the gel migrate slightly slower than the proteins on the other face. This causes the bands to slant downward from the cooled to uncooled face across the thickness of the gel. Although bandwidths may be the same as without cooling, the protein bands can appear broadened when they are viewed face on. The solution to this problem is to not apply cooling to the cell.

So-called mini-cells and midi-cells allow rapid analysis and are adequate for relatively uncomplicated samples. The design of these cells allows runs to be completed in as little as 35-45 min. Mini-gels are about 7 cm long by 8 cm wide while midi-gels are of the order of 15 x 10 cm. They are both very easy to handle. Some midi-gels can hold up to 26 samples.

All three size-categories of gels can be used for nearly all purposes. The resolution that can be obtained between protein bands is the same for mini-, midi-, and maxi-gels. However, the separation between bands, as defined in Section 5, is greater with longer gels. Closely spaced bands are easier to distinguish from one another, their bandwidths are easier to measure, and they are more easily cut out from a large gel than from a small one.

4.1 Electrical considerations

Regulated direct current (DC) power supplies, designed for electrophoresis, allow control of every electrophoretic mode. Constant voltage, constant current, or constant power conditions can be selected. Many power supplies have timers and some have integrators allowing runs to be automatically terminated after a set time or number of volt-hours. All modes of operation can produce satisfactory results, but for best results and good reproducibility, some form of electrical control is important. The choice of which electrical parameter to control is almost a matter of preference. The major limitation is the ability of the chamber to dissipate the heat generated by the electrical current.

During an electrophoresis run, electrical energy is converted into heat, called Joule heat. This heat can have many deleterious consequences, such as band distortion, increased diffusion, enzyme inactivation, and protein denaturation. All good
electrophoresis chambers are designed to transfer the heat generated in the gel to the outside environment. In general, electrophoresis should be carried out at voltage and current settings at which the run proceeds as rapidly as the ability of the chamber to draw off heat allows. That is, the run should be as fast as possible without exceeding desired resolution and distortion limits - and these can only be determined empirically for any given system. Each experiment will impose its own criteria on cooling efficiency. Nearly all electrophoresis runs can be carried out on the laboratory bench, but some delicate proteins may require that the runs be conducted in the cold room or with circulated coolant.

Electrical quantities are interrelated by fundamental laws. Each gel has an intrinsic resistance, \( R \), determined by the ionic strength of its buffer (\( R \) changes with time in discontinuous systems). When a voltage \( V \) is impressed across the gel a current \( I \) flows through the gel and the external circuitry. Ohm’s law relates these three quantities: \( V = IR \), where \( V \) is expressed in volts, \( I \) in amperes, and \( R \) in ohms. In addition, power \( P \), in watts, is given by \( P = IV \). The generation of Joule heat, \( H \), is related to power by the mechanical equivalent of heat, 4.18 J/cal, or \( H = (P/4.18) \text{ cal/sec} \).

With continuous buffer systems, the resistance of the gel is essentially constant, although it will decrease a bit during a run as the buffer warms. With the discontinuous Ornstein-Davis or Laemmli buffers, \( R \) increases during the course of a run as the chloride ions are exchanged by glycinate. At constant current in Laemmli gels, the voltage, power \( (I^2R) \), and consequently the heat generated in the gel chamber increase during the run. Under constant voltage conditions, current, power \( (V^2/R) \), and heat generation decrease during electrophoresis as \( R \) increases. Voltage and current should be set to keep \( H \) below the dissipation limit of the electrophoresis chamber. Follow the recommendations of the manufacturer for the proper electrical settings to use with any particular cell. Vertical cells are usually run at electric field strengths of 10-20 V/cm or currents in the range of 15-25 mA/mm of gel thickness.

The voltage applied to an electrophoresis cell is divided across three distinct resistance regions. The buffer paths from the open ends of the gel to the electrode wires form two of these regions. These two resistance regions are usually ignored but they should be kept in mind for electrical analysis when experimenting with electrode buffers having very high or very low conductivity. The gel buffer is the third resistance region. With the Laemmli SDS system, the buffers create two different resistive sections. The low-resistance leading \( \Gamma^- \) ion forms a resistance segment that runs ahead of the higher-resistance trailing glycinate ion segment. Taken together, the two gel-segment resistors act as a voltage divider. The voltage across either one of the gel segments is proportional to the resistance of that segment. The voltage across the chloride section provides the force that pulls the ion front through the gel, whereas the voltage across the glycinate section pulls the proteins through the gel. This proportioning of the applied voltage can cause two gels of the same %T to run differently if their gel buffers are different. For example, the final band pattern in a 12%T Laemmli gel with a gel buffer at pH 8.6 looks like the band pattern of a 10%T Laemmli gel with a gel buffer at pH 8.8. The gel at pH 8.6 also takes about 20% longer to run than the gel at pH 8.8. The differences in the properties of the two gel types are due to the increased conductivity of the pH 8.6 gel relative to the pH 8.8 gel. The extra chloride needed to drop the pH of 0.375 M Tris from 8.8 to 8.6 brings about the increased conductivity (0.12 M Cl⁻ vs. 0.19 M Cl⁻). A subtle
5. Resolution and separation

Evaluations of electrophoretic data usually include mention of separation or resolution. Although the two terms are not synonymous they are often treated as such. Because of the way in which people visualize gels, separation may be the more important of the two terms for electrophoresis. Both large and small gels can have the same resolution, but there will be more space between the bands of larger gels. This added “landscape” of larger gels is reassuring to researchers and makes the excision of bands from large gels easier to accomplish than from small gels.

Separation refers to the distance between two adjacent bands. The eye tends to see bands as being sharply defined with clearly evident blank spaces between adjacent bands. Thus, for practical purposes, separation should be taken to be the distance between the top of the faster running of two adjacent bands and the bottom of the slower one. It is the distance between the top of the bottom band and the bottom of the top band. This definition seems preferable in electrophoresis to defining separation as the distance between band centers.

Resolution, on the other hand, is a more technical term. It refers to the distance between adjacent bands relative to their bandwidths and acknowledges the fact that proteins are distributed in gaussian profiles with overlapping distributions between bands. The numerical expression for resolution is obtained by dividing the distance between the centers of adjacent bands by some measure of their average bandwidths. It expresses the distance between band centers in units of bandwidth and gives a measure of the overlap between two adjacent bands. For preparative applications, when maximal purity is desired, the length of at least one bandwidth should separate two bands. In other applications, it may be sufficient to be able to simply discern that two bands are distinct. In this latter case, the bands can be less than a bandwidth apart. Several software packages are available that use gaussian modeling to differentiate and quantify bands for the quantitative analysis of gel images.

6. Detergents

Detergents are employed in electrophoresis when it is necessary to disrupt protein-lipid and protein-protein interactions. A variety of detergents has been used for this purpose (39, 40).

SDS is the most common detergent used in PAGE analysis. Most proteins are readily soluble in SDS, making SDS-PAGE a generally applicable method. In SDS-PAGE, the quality of the SDS is of prime importance. The effects of impurities in SDS are unpredictable. Of the contaminants, the worst offenders are probably the alkyl sulfates other than dodecyl sulfate (C_{12}); especially decyl sulfate (C_{10}), tetradecyl sulfate (C_{14}), and hexadecyl sulfate (C_{16}) (41, 42). These bind to proteins with different affinities, thereby affecting mobilities. Lipophilic contaminants in SDS preparations, including dodecanol, can be trapped in SDS-protein complexes and SDS micelles, leading to loss of resolution. Only purified SDS should be used for electrophoresis, but even with pure SDS, various glycoproteins, lipoproteins, and nucleoproteins tend to bind
the detergent irregularly. The resultant SDS-polypeptides then migrate “anomalously”
with respect to their molecular masses.

Several types of proteins do not behave as expected during SDS-PAGE (1, 34).
Incomplete reduction, which leaves some intra- or intermolecular disulfide bonds intact,
makes some SDS-binding domains unavailable to the detergent so that the proteins are
not saturated with SDS. Glycoproteins and lipoproteins also migrate abnormally in SDS-
PAGE, because their non-proteinaceous components do not bind the detergent uniformly.
Proteins with unusual amino acid sequences, especially those with high lysine or proline
content, very basic proteins, and very acidic proteins behave anomalously in SDS-PAGE,
variably because the charge-to-mass ratios of the SDS-polypeptide complexes are
different than those that would be expected from size alone. Similarly, very large SDS-
proteins, with molecular masses in the several hundred-kilodalton range, may have
unusual conformations. Polypeptides smaller than about 12,000 Da are not resolved well
in most SDS-PAGE systems. In most cases, they do not separate from the band of SDS
micelles that forms behind the leading ion front. The Tricine buffer system was devised
to separate these small polypeptides.

7. Choice of system
7.1 Native proteins

Continuous buffer systems are preferred for native work because of their
simplicity. Furthermore, some native proteins may aggregate and precipitate at the very
high protein concentrations reached during stacking in discontinuous electrophoresis.
Consequently, either they might not enter the resolving gel or they might cause streaking
as accumulated protein slowly dissolves during a run. If the proteins of interest behave in
this manner, it is best to use some form of continuous buffer system.

The pH of the electrophoresis buffer must be in the range over which the proteins
of interest are stable or where they retain their biological activity. The pH should also be
properly chosen with respect to the isoelectric point (pI). The pH of the gel buffer should
be far enough away from the pIs of the proteins of interest that they carry enough net
charge to migrate through the gel in a reasonable time. On the other hand, separation
of two proteins at a given gel concentration is best near one of their isoelectric points,
because the isoelectric protein will barely move in that pH range. (Figure 4 shows how
the buffer choice determines migration rates.) The choice of pH is often a compromise
between considerations of resolution and stability. For best results with continuous
systems, the concentrations of the proteins of interest should be at least 1 mg/ml to keep
sample volume at a minimum. The sample should be loaded in gel buffer diluted to ½ to
1/5 strength (some form of buffer exchange may be required). The decreased ionic
strength of diluted buffer causes a voltage to develop across the sample that assists in
driving the proteins into the gel.

The choice of proper gel concentration (%T) is, of course, critical to the success
of the separation, since it heavily influences separation. Too high %T can lead to
exclusion of proteins from the gel and too low %T can decrease sieving (see Figure 3).
One approach, useful with the McLellan continuous buffers (Table 1), is to use relatively
large-pore gels (5%T to 7%T) and to alter mobilities with pH (Protocol 1 uses 6%T). An
approach for discontinuous systems is to start with a 7.5%T gel, then, if that is not
satisfactory, to try a number of gel concentrations between 5%T and 15%T. Pore-gradient gels can also be tried.

### 7.2 Denatured proteins

It is easier to choose suitable gel concentrations (%T) for SDS-PAGE than for native protein gels because the separation of SDS-polypeptides is dependent mainly on chain length. Laemmlı gels with 7.5%T resolve proteins in the 40- to 200-kDa range, those with 10%T resolve 20- to 200-kDa proteins, 12%T gels separate proteins in the 15- to 100-kDa range, and 15% gels separate 6- to 90-kDa proteins (Figure 6).

<table>
<thead>
<tr>
<th>5%</th>
<th>7.5%</th>
<th>10%</th>
<th>12.5%</th>
<th>15%</th>
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<th>1%</th>
<th>15%</th>
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<td>15</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

**Figure 6.** Protein migration charts. Relative positions of standard proteins are shown for several SDS-PAGE gels of the Laemmlı type (“Tris-HCl”) and two Tricine SDS-PAGE gels (“Peptide”).

### 8. Sample preparation

Samples for SDS-PAGE by the Laemmlı procedure are prepared in diluted gel buffer containing SDS, 2-mercaptoethanol, glycerol, and bromophenol blue tracking dye (Table 3). It is best to prepare a stock solution of sample buffer containing everything but 2-mercaptoethanol and to add this reagent right before use. The glycerol provides density for applying the sample on the stacking gel under electrode buffer. The tracking dye allows both sample application and the electrophoretic run to be monitored (it migrates with the ion front). There is sufficient SDS present in the sample buffer to ensure saturation of most protein mixtures (31). Except in the rare instances when the sample is in a very high-ionic-strength solution (>0.2 M salts), it can be dissolved 1:1 (v/v) in stock sample buffer. It is much better, though, to dilute the sample at least 1:4 (v/v) with the sample buffer stock. The amount of sample protein to load on a gel depends on the detection method to be used (see the Application Focus about the Detection of Proteins in Gels on this website). Enough of the protein of interest must be loaded on the gel for it to be subsequently located. Detection in gels requires on the order of 1 μg of protein for easy visibility of bands stained with anionic dyes such as Coomassie Brilliant Blue R-250
or 0.1 µg of protein with silver staining. Complete dissociation of most proteins is achieved by heating diluted samples to 95-100°C for 2-5 minutes.

**Table 3. Compositions of sample buffers**

**McLellan native, continuous buffer systems**

<table>
<thead>
<tr>
<th>0.5X gel buffer, 10% (w/v) glycerol, 0.01% bromophenol blue</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5X Gel buffer&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>50% Glycerol&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>0.5% Bromophenol blue&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Water</td>
<td>6.8 ml</td>
</tr>
</tbody>
</table>

Store at room temperature.

**Ornstein-Davis native, discontinuous buffer**

<table>
<thead>
<tr>
<th>0.0625 M Tris-Cl, pH 6.8, 10% glycerol, 0.01% bromophenol blue</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stacking gel 4X buffer stock&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.25 ml</td>
</tr>
<tr>
<td>50% (w/v) Glycerol&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>0.5% Bromophenol blue&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Water</td>
<td>6.55 ml</td>
</tr>
</tbody>
</table>

Store at room temperature.

**Laemmli SDS-PAGE buffer**

<table>
<thead>
<tr>
<th>0.075 M Tris-Cl, pH 8.8, 2% SDS, 5% 2-mercaptoethanol, 10% glycerol, 0.01% bromophenol blue</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock sample buffer</td>
<td></td>
</tr>
<tr>
<td>Resolving gel 4X buffer stock&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>10% SDS&lt;sup&gt;f&lt;/sup&gt;</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>50% (w/v) Glycerol&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0 ml</td>
</tr>
<tr>
<td>0.5% Bromophenol blue&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Water</td>
<td>4.8 ml</td>
</tr>
</tbody>
</table>

Store at room temperature. Prior to use, add 50 µl of 2-mercaptoethanol to 950 µl of sample buffer stock. An alternative sample buffer that is often used is obtained by adjusting the Ornstein-Davis sample buffer to 2% SDS and 5% 2-mercaptoethanol.

**Tricine SDS-PAGE sample buffer**

<table>
<thead>
<tr>
<th>0.1 M Tris-Cl, pH 8.45, 1% SDS, 2% 2-mercaptoethanol, 20% glycerol, 0.04% CBB G-250</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock sample buffer</td>
<td></td>
</tr>
<tr>
<td>Tricine gel 3X buffer stock&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.33 ml</td>
</tr>
<tr>
<td>10% SDS&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1 ml</td>
</tr>
<tr>
<td>50% (w/v) Glycerol&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4 ml</td>
</tr>
<tr>
<td>0.5% CBB G-250&lt;sup&gt;h&lt;/sup&gt;</td>
<td>0.4 ml</td>
</tr>
<tr>
<td>Water</td>
<td>3.77 ml</td>
</tr>
</tbody>
</table>

Store at room temperature. Prior to use, add 20 µl of 2-mercaptoethanol to 980 µl of buffer.

<sup>a</sup>5X McLellan gel buffer. See Table 1.

<sup>b</sup>50% (w/v) Glycerol. See Protocol 3.

<sup>c</sup>0.5% Bromophenol blue. Dissolve 50 mg of bromophenol blue in 10 ml of water.

<sup>d</sup>Stacking gel 4X buffer stock. See Protocol 2.

<sup>e</sup>Resolving gel 4X buffer stock. See Protocol 2.

<sup>f</sup>10% SDS. Dissolve 10 g of SDS in ~70 ml of water, then adjust the volume to 100 ml.
Tricine gel 3X buffer stock. See Protocol 3.

0.5% Coomassie Brilliant Blue G-250. Dissolve 50 mg of CBB G-250 in 10 ml of water.

For native, discontinuous gels, upper gel buffer diluted twofold to fivefold for sample application is commonly used. Tracking dye and glycerol are added to these samples also, and protein concentrations should fall within the same limits as for SDS-PAGE. With discontinuous systems, the volume of sample is not very important as long as the height of the stacking gel is at least twice the height of the sample volume loaded on the gel. Continuous systems require minimal sample volumes for best resolution.

Careful sample handling is important when sensitive detection methods are employed. Silver-stained SDS-PAGE gels sometimes show artifact bands in the 50- to 70-kDa molecular mass region and irregular but distinctive vertical streaking parallel to the direction of migration. The appearance of these artifacts has been attributed to the reduction of contaminant skin keratin inadvertently introduced into the samples (43). The best remedy for the keratin artifact is to avoid introducing it into the sample in the first place. Monomer solution, stock sample buffer, gel buffers, and upper electrode buffer should be filtered through nitrocellulose and stored in well-cleaned containers. It also helps to clean the gel apparatus thoroughly with detergent and to wear gloves while assembling the equipment.

Prepared samples are placed in sample wells of gels with microliter syringes or micropipettes. Both types of liquid-handling device provide good control of sample volume. Syringes must be thoroughly rinsed between applications to avoid cross contamination of different samples. Standard pipette tips are too wide to fit into narrow sample wells, but several thin tips, specifically designed for sample application, are available. The choice of sample-loading device is one of personal preference.

Protocol 4. Sample preparation and electrophoresis

Equipment and reagents
- Gel electrophoresis apparatus; e.g., the Bio-Rad Mini-PROTEAN 3.
- Electrophoresis power supply capable of delivering at least 300 V and 200 mA; e.g., the Bio-Rad PowerPac 300 or PowerPac 1000.
- Electrode buffer. See Table 2. Consult the apparatus instructions for the volume required.
- Sample buffer. See Table 3.

Method
1. Assemble the electrophoresis apparatus and insert the gels according to the manufacturer’s instructions. With some electrophoresis cells, it is sometimes easier to load the wells before the gel assembly is placed in the buffer tank.
2. Remove the combs from the gels and rinse the wells with electrode buffer.
3. Fill the upper reservoir of the electrophoresis cell so that buffer fills the wells.
4. Mix one volume of protein sample with at least two volumes of the appropriate sample buffer (see Table 3). Prepare protein standards or markers in the same manner as the samples. Sample volumes depend on the types of proteins, the type
of detection, and the size of the sample wells. Volumes loaded into the wells are often of the order of tens of microliters.

5. Heat samples for SDS-PAGE (Laemmli or Tricine) at 95°C for 2 min in a temperature block or water bath. (Make sure that 2-mercaptoethanol is included in the sample buffer for SDS-PAGE. See Table 3.) Do NOT heat samples for non-denaturing conditions (McLellan or Ornstein-Davis systems).

6. Load the samples carefully in the wells of the gels with a microsyringe or micropipette; e.g., Bio-Rad Prot/Elec pipette tips. The glycerol in the sample buffers (Table 3) provides density for layering the samples under the buffer in the wells.

7. Place the gel assembly in the lower-electrode buffer tank, if sample loading was done outside the tank. Add electrode buffer to the electrode chambers as necessary. It is often unnecessary to immerse the gels completely in buffer.

8. Put the lid on the tank and connect the leads to the power supply. For most gel types, the anode (+) (red lead) is at the bottom of the tank and the cathode (-) (black lead) is at the top. However, it is necessary to reverse the leads with CTAB gels and with the high pH McLellan gels. For safety, power supplies for electrophoresis should have with isolated grounds.

9. Turn on the power supply and set it for the electrical conditions recommended by the manufacturer of the electrophoresis cell; e.g., 200 V constant voltage for Laemmli gels run in the Bio-Rad Mini-Protean 3.

10. Allow the run to proceed until the blue tracking dye from the sample buffer reaches the end of the gel (about 40 min for Laemmli gels in the Mini-PROTEAN 3). It is not necessary to remove the gel immediately at the end of a run. Gels can be left for extended periods – at least overnight – without deterioration of the band patterns.

9. Molecular mass estimation

SDS-PAGE has become the most popular method of gel electrophoresis because it can be used to estimate molecular masses (1, 3, 8, 34, 44). To a first approximation, migration rates of SDS polypeptides are inversely proportional to the logarithms of their molecular masses. The larger the polypeptide, the slower it migrates in a gel.

Molecular masses are determined in SDS-PAGE by comparing the mobilities of test proteins to the mobilities of known protein markers. At one time, when samples for SDS-PAGE were run in individual tubes, it was necessary to normalize to a common parameter so that the different tube gels could be compared. This was because tube gels differ in length. The normalizing parameter that is still used is the relative mobility, \( R_i \), defined as the mobility of a protein divided by the mobility of the ion front. In practice, when all gels are run for the same length of time, \( R_i \) is calculated as the quotient of the distance traveled by a protein from the top of the resolving gel divided by the distance migrated by the ion front. The distance to the ion front is usually taken as the distance to the tracking dye (measured or marked in some way before staining). With slab gels, this normalization is less important provided that a lane of standards is run in the same gel as the samples whose masses are to be determined. It is sufficient to compare migration
distances of samples and standards. Plots of the logarithm of protein molecular mass (log \( M_r \)) versus the migration distances fit reasonably straight lines.

In each gel, a lane of standard proteins of known molecular masses is run in parallel with the test proteins. After staining the gel to make the protein bands visible (see the Application Focus about the Detection of Proteins in Gels on this website), the migration distances are measured from the top of the resolving gel. The gel is calibrated with a plot of log \( M_r \) vs. migration distances for the standards. The migration distances of the test proteins are compared with those of the standards. Interpolation of the migration distances of test proteins into the standard curve gives the approximate molecular masses of the test proteins.

Pore-gradient SDS-PAGE gels can also be used to estimate molecular masses. In this case, log \( M_r \) is proportional to log (%T). With linear gradients, %T is proportional to distance migrated, so that the data can be plotted as log \( M_r \) vs. log (migration distance).

Standard curves are actually sigmoid in shape (Figure 7). The apparently linearity of a standard curve may not cover the full range of molecular masses for a given protein mixture in a particular gel. However, log \( M_r \) is sufficiently slow, in a mathematical sense, to allow fairly accurate molecular mass estimates to be made by interpolation, and even extrapolation, over relatively wide ranges. The approximate useful ranges of single-percentage SDS-PAGE gels for molecular mass estimations is as follows: 40,000 to 200,000 Da, 7.5%T; 30,000 to 100,000, 10%T; 15,000 to 90,000 Da, 12%T; 10,000 to 70,000 Da, 15%T. Mixtures of standard proteins with known molecular masses are available commercially for calibrating electrophoresis gels.
Figure 7. A representative calibration curve for molecular mass estimation. In the run that is plotted here (solid line), Commercial SDS-PAGE Standards with molecular masses of 200, 116.2, 97.4, 66.2, 45, 31, 21.5, 14.4, and 6.5 kDa (top to bottom, closed circles) were separated in an 15%T SDS-PAGE gel. The plot of \( \log_{10}(M, \times 10^{-3}) \) vs. Relative Mobility \( R_i \) shows the inherent non-linearity of such curves. The straight-line segment in the middle of the plot is the most accurate range for molecular mass estimations. Larger polypeptides experience greater sieving than do those in the middle range; the upper part of the curve has a different slope than in the middle. Small polypeptides experience less sieving than the others and also deviate from a strictly straight-line dependence. It is customary to estimate molecular masses from a “best fit” straight line (dashed). This is sufficient for some purposes and is acceptable because the logarithm function changes slowly with its argument.

The semi-logarithmic plots used for molecular mass determinations are holdovers from the days when people had only graph paper and straight edges for curve fitting. (From a mathematical point of view, \( \log M_i \) should be the independent variable (x-axis) and migration distance should be the dependent variable (y-axis). Not as usually drawn.) Several computer programs allow for standard curves to be fit with different mathematical functions than the semi-logarithmic model. Some other types of curves actually fit the data better than the semi-log function. Nevertheless, the semi-logarithmic model for standard curves is the accepted norm.

It is important to bear in mind that the molecular masses obtained using Laemmli SDS-PAGE are those of the polypeptide subunits and not those of native, oligomeric proteins. Moreover, proteins that are incompletely saturated with SDS, very small polypeptides, very large proteins, and proteins conjugated with sugars or lipids behave
anomalously in SDS-page, as mentioned above. Nevertheless, SDS-PAGE provides reasonable molecular mass estimates for most proteins.

10. Precast Gels
The biggest change in gel electrophoresis since the advent of polyacrylamide gels in the 1960’s is the commercial availability of precast gels. Since the early 1990’s, several companies have made a wide variety of precast polyacrylamide gels available to the research community. Most of the precast gels offered are Laemmli SDS-PAGE gels of differing %T and numbers of wells. Because the Laemmli SDS-PAGE gel is so overwhelmingly popular, alternative types of electrophoresis gels have tended to be ignored by researchers and the companies have focused on this bias. In fact, it is probably safe to state that many people do not know that there is any other type of protein gel electrophoresis than the Laemmli method.

It took more than 20 years for manufacturers to devise production and distribution networks for delivering consistently high quality gels to customers. The problems that the companies faced stem from the limited shelf life of polyacrylamide gels. Because polyacrylamide gels hydrolyze over time as shown in Figure 1, they are inherently unstable. The shelf life of a gel cast in the Laemmli gel buffer (pH 8.8) is about 3-4 months. It is not possible to cast large volumes of gels and to hold them in a warehouse for long periods of time. A great deal of planning goes into the decisions of how many gels of each different type are to be cast at any particular time. Manufacturing and distribution issues have now been largely addressed and customers can now be guaranteed that they will receive gels that can be stored for several weeks before they are used. A limited number of precast gel products are available that are cast with neutral pH buffers (45). These gels have longer shelf lives than gels made according to the Laemmli formulation. However, since the band patterns obtained with neutral pH gels are different than those obtained with Laemmli gels, they have not found universal acceptance.

People were initially drawn to precast gels by the gradients. It is more appealing to be able to buy gradient gels that are already made than to cast them. The ease in use of precast gradient gels led to the acceptance of single percentage gel types as well. For all but the most demanding situations there is little reason to cast gels by hand. The gel types most in demand are 7.5%T, 10%T, 12%T, 4-15%T, and 4-20%T.

Precast gels differ from hand cast gels in three ways. They are cast with a single buffer throughout, without SDS, and without a sharp demarcation between the stacking and resolving gels. As pointed out previously, because SDS dominates the system, using different buffers in the stacking and resolving gels as in the original Laemmli formulation has no practical value. The two different buffers would mingle together on storage without elaborate means to keep them separate. In addition, during electrophoresis SDS from the cathode buffer sweeps past the proteins in the resolving gel and keeps them saturated with SDS even when there is no SDS in the gel when it is cast. Precast gels are thus made without SDS. This is beneficial to both the manufacturer and the user. SDS tends to form bubbles in the pumping systems used to deliver monomer solutions to gel cassettes, causing problems with monomer delivery. It also forms micelles that can trap acrylamide monomer and lead to heterogeneity of pore size.

When gels are cast by hand, it is customary to allow the resolving gel to harden before the stacking gel is placed on top of it. This practice is acceptable since hand cast
Gels are usually used within a short period of time. On the other hand, when gels are cast this way and stored, the stacking gel eventually begins to pull away from the resolving gel. The gap that forms between the two gels leads to lateral spreading of the stacked proteins and destruction of the stack as it leaves the upper gel. For this reason, precast gels are cast in a continuous manner with the stacking gel monomer solution added on top of the resolving gel monomer solution before gelation. This means that the separation between the two gel types is a gradual one rather than a sharp one. Even though the distance between the two gels is short, the transition between gel types exists as a short gradient of %T. Proteins “unstack” gradually rather than abruptly. Because of this, the bands obtained with precast gels are not quite as sharp as those obtained with hand cast.

11. References