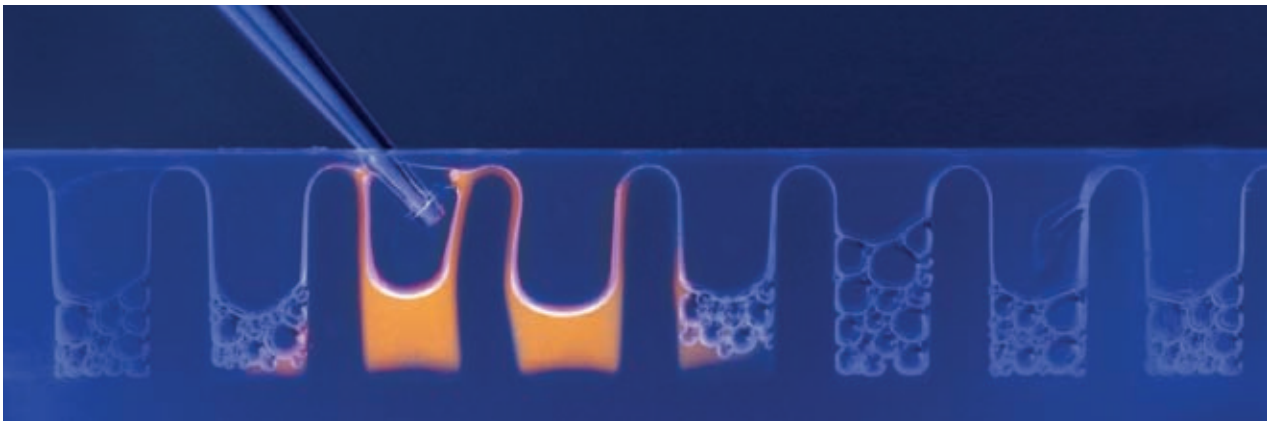


In This Section

Overview

Polyacrylamide gel electrophoresis (PAGE) is a powerful tool for separating and identifying mixtures of proteins and peptides. Several systems exist for performing PAGE and consideration should be given to which system best suits a given sample prior to running the samples.

Gradient vs. homogeneous (straight percentage) gels

Gradient gels are suitable for a wide range of size resolutions, and will result in tighter band separation than single concentration gels. A homogeneous, or single concentration gel is appropriate where the proteins of interest are known to be within a narrow size range.

Discontinuous and continuous buffer systems

A discontinuous buffer system utilizes a large-pore gel (the stacking gel) layered on top of a small pore gel (the resolving gel). In this system, the buffer used to prepare the gels is different from the buffer used in the tank. The different buffers create two ion fronts concentrating the proteins into a very tight zone.

A continuous buffer system uses the same buffer in the gel and the tank. This system uses a single separating gel (no stacking gel) and has a single ion front. This system is less used than discontinuous systems, however, the separation of specific proteins and protein complexes that precipitate or aggregate may require the use of a continuous system.

Buffers for Protein Electrophoresis

The Laemmli Buffer System (Tris-Glycine) is a discontinuous buffer system, widely used for fine resolution of a broad molecular weight range of proteins. In this system, the gel is prepared with Tris-HCl Buffer and the Tris-glycine is used as the running buffer.

In the Tris-Tricine Buffer System, tricine replaces glycine in the running buffer. The result is more efficient stacking and destacking, and higher resolution of proteins and peptides with lower molecular weights (under 10 kDa - 15 kDa).

Buffer Preparation Tris-Glycine SDS Buffer, pH 8.3	
10X Stock Solution	**g/l for 10X Stock Solution**
.25 M Tris base	30.3 g Tris Base
1.92 M Glycine	144.0 g Glycine
1.0% SDS*	10.0 g SDS
	Adjust volume to 1 liter with distilled water

(1X = 25 mM Tris base, 192 mM Glycine, 0.1% SDS*)
*Omit SDS if running native proteins.

Tris-Tricine SDS Buffer, pH 8.3	
10X Stock Solution	**g/l for 10X Stock Solution**
1 M Tris base	121.1 g Tris base
1 M Tricine	179.0 g Tricine
1.0% SDS*	10.0 g SDS
	Adjust volume to 1 liter with distilled water

(1X = 100 mM Tris base, 100 mM Tricine, 0.1% SDS*)
*Omit SDS if running native proteins.

Casting Polyacrylamide Gels

Follow the steps below to cast a straight percentage polyacrylamide gel with a stacking gel. Alternatively, Lonza offers ready-to-use ProSieve® 50 Gel Solution, or PAGEr® Gold Precast Gels in a wide variety of concentrations and well formats.

Casting the resolving gel

1. Assemble plates according to the manufacturer's instructions.
2. Place the specified quantity of the first four components from the table on page 175 into a side arm flask.
3. Mix gently by swirling.

NOTE: Omit SDS if running a native gel.

4. Degas the solution for 15 minutes. A high concentration of APS (0.3%) can be used to speed polymerization and skip the degassing step.
5. Add the specified amounts of TEMED and 10% APS.
6. Mix gently by swirling.
7. Pour the resolving gel, leaving space for the stacking gel.
8. Gently overlay the acrylamide with water-saturated isobutanol. The overlay blocks oxygen from inhibiting polymerization of the resolving gel.
9. Allow the gel to polymerize for 30 - 60 minutes. A very sharp liquid-gel interface will be visible when the gel has polymerized.
10. Pour off the overlay.
11. Rinse the top of the gel several times with water.
12. Blot any remaining water with a paper towel.

- Materials
  - Vertical electrophoresis plate assembly with comb
  - Sidearm flask assembly or filter unit for degassing solutions
  - Paper towels
- Reagents
  - 30% Acrylamide mix, 29% (w/v) Acrylamide, 1% (w/v) bisacrylamide, prepared in distilled water
  - 1.5 M Tris Buffer pH 8.8
  - 10% SDS
  - 10% Ammonium Persulfate (APS)
  - TEMED
  - Distilled water
  - Water saturated isobutanol

Caution: Wear gloves, safety glasses and lab coats when handling acrylamide solutions.

Preparation of Tris-Glycine Resolving Gel

Solution components	Component volumes (ml) 10 ml	20 ml	50 ml
6%			
Distilled water	5.3	10.6	26.5
30% Acrylamide mix	2.0	4.0	10.0
1.5 M Tris (pH 8.8)	2.5	5.0	12.5
10% SDS	0.1	0.2	0.5
10% Ammonium persulfate	0.1	0.2	0.5
TEMED	0.008	0.016	0.04
8%			
Distilled water	4.6	9.3	23.2
30% Acrylamide mix	2.7	5.3	13.3
1.5 M Tris (pH 8.8)	2.5	5.0	12.5
10% SDS	0.1	0.2	0.5
10% Ammonium persulfate	0.1	0.2	0.5
TEMED	0.006	0.012	0.03
10%			
Distilled water	4.0	7.9	19.8
30% Acrylamide mix	3.3	6.7	16.7
1.5 M Tris (pH 8.8)	2.5	5.0	12.5
10% SDS	0.1	0.2	0.5
10% Ammonium persulfate	0.1	0.2	0.5
TEMED	0.004	0.008	0.02
12%			
Distilled water	3.3	6.6	16.5
30% Acrylamide mix	4.0	8.0	20.0
1.5 M Tris (pH 8.8)	2.5	5.0	12.5
10% SDS	0.1	0.2	0.5
10% Ammonium persulfate	0.1	0.2	0.5
TEMED	0.004	0.008	0.02
15%			
Distilled water	2.3	4.6	11.5
30% Acrylamide mix	5.0	10.0	25.0
1.5 M Tris (pH 6.8)	2.5	5.0	12.5
10% SDS	0.1	0.2	0.5
10% Ammonium persulfate	0.1	0.2	0.5
TEMED	0.004	0.008	0.02

NOTE: Adjust proportionally based on the amount of gel needed.

Preparation of a 5% stacking gel

1. Place the specified quantity of the first four components from the table below into a side arm flask.

Component volumes (ml)

Solution components	1 ml	5 ml	10 ml
Distilled water	0.68	3.4	6.8
30% Acrylamide mix	0.17	0.83	1.7
1.5 M Tris (pH 8.8)	0.13	0.63	1.25
10% SDS	0.01	0.05	0.1
10% Ammonium persulfate	0.01	0.05	0.1
TEMED	0.001	0.005	0.01

2. Mix gently by swirling.

NOTE: Omit SDS if running a native gel.

3. Degas the solution for 15 minutes.
4. Add the specified amounts of TEMED and 10% APS.
5. Mix gently by swirling
6. Pour the stacking gel directly onto the resolving gel.
7. Insert the comb immediately.
8. Allow the gel to polymerize for at least one hour.
9. Prepare samples while the gel is polymerizing.
10. Carefully remove the comb.

NOTE: Gels may be stored overnight at 4°C with the comb in place and wrapped in plastic wrap.

Casting gradient polyacrylamide gels

Hand casting gradient gels is not covered in this resource. Due to the complexity involved in hand casting gradient gels, precast gels have become a popular alternative. We recommend PAGEr® Precast Gels (see page 48), or ProSieve® Gel Solution (see page 60), which provides gradient quality separation from a linear gel format. Detailed discussions and protocols for preparing gradient gels can be found in *Electrophoresis in Practice,* 2nd Edition.

Introduction

Protein load levels will vary depending upon sample purity and staining method used. For highly purified proteins, 0.5 µg to 5 µg protein per lane on a minigel is generally sufficient. Complete mixtures such as cell lysates may require as much as 50 µg protein per lane. The table below provides lower detection limits for protein detection.

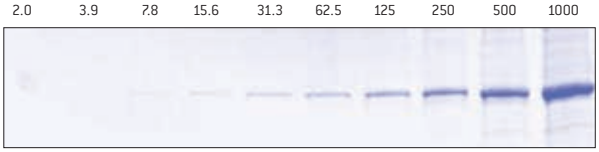
Protein stain detection limits

Protein Stain	Lower Detection Limit (protein/band)
Coomassie® Blue Stain	100 ng
Silver Stain	1 ng
SYPRO® Orange Protein Gel Stain	1 ng–2 ng
SYPRO® Red Protein Gel Stain	1 ng–2 ng
SYPRO® Tangerine Protein Gel Stain	4 ng–8 ng

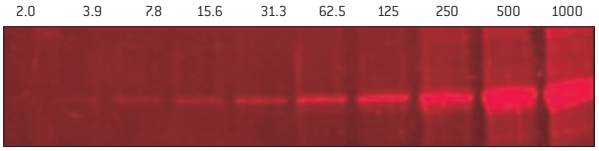
NOTE: Limits are based on optimal detection methods for each stain.

The photographs below demonstrate the detection sensitivities of commonly used stains

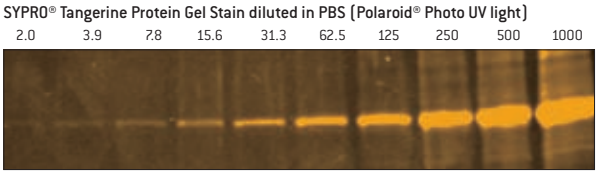
Coomassie® Blue Stain (CCD camera)



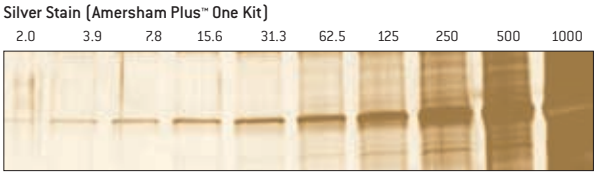
SYPRO® Red Protein Gel Stain diluted in 7.5% acetic acid (Polaroid® Photo UV light)



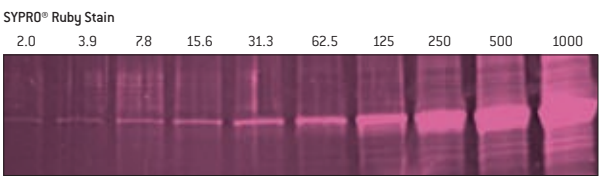
SYPRO® Tangerine Protein Gel Stain diluted in PBS (Polaroid® Photo UV light)



Silver Stain (Amersham Plus™ One Kit)



SYPRO® Ruby Stain



Serial dilutions of ProSieve® Protein Markers were separated on 12% PAGEr® Gold Precast Gels, stained and photographed as noted. The figure shows the staining sensitivity seen for the 50 kDa band of the marker. The level of protein present is indicated in nanograms. Staining was performed following the manufacturer's instructions provided with the stains. Exposure times for photography were adjusted to obtain highest possible detection levels. Note that in both silver stain examples shown, development was allowed to proceed for an extended time period relative to the range given in the instructions to maximize detection. In the case of the Amersham kit; this results in overexposure of higher protein loading levels. Images of the silver stained gels were captured using a CCD camera system.

Introduction

In general, loading buffers for protein electrophoresis contain Tris-HCl, pH 6.8; 2% SDS; a reducing agent such as dithiothreitol (DTT), β-mercaptoethanol (βME), or Tris[2-Carboxyethylphosphine]hydrochloride (TCEP); glycerol (a sinking agent) and a marker dye. An alternative reducing agent to βME, Bond-Breaker™ TCEP Solution from Thermo Scientific is an odor-free, ready-to-use solution added to the sample buffer prior to denaturation.

Gel loading buffers serve four purposes in protein electrophoresis:

— Reduction of protein complexes if performing denaturing PAGE

— Dissociation of proteins to allow them to run through the gel

— Increase the density of the sample ensuring samples drop evenly into the well

— Addition of a dye to the sample to simplify loading and monitor the electrophoretic process

2X Tris-Glycine SDS sample buffer	
2X concentrate	amount to add for 2X concentrate
126 mM Tris-HCl, pH 6.8	2.5 ml of 0.5 M Tris-HCl, pH 6.8
20% Glycerol	2 ml Glycerol
4% SDS	4 ml of 10% SDS
0.005% Bromophenol blue	0.5 ml of 0.1% Bromophenol blue
Adjust volume to 10 ml with distilled water	

(1X = 63 mM Tris-HCl, 10% glycerol, 2% SDS, 0.0025% Bromophenol blue, 2.5% βME)

Sample preparation tips for sample preparation

— Keep samples on ice prior to adding the sample buffer

— Add room temperature sample buffer to the cold samples

— If preparing samples for future use, aliquot treated samples into usable aliquots to avoid freeze thawing

— Do not leave the samples in SDS sample buffer at room temperature without first heating to 95°C to inactivate proteases. A loss of high-molecular weight bands and general smearing of the bands are indications of protease activity

Procedure for sample preparation

Follow the guidelines below for preparing protein samples for electrophoresis.

1. Add 0.5 ml βME or 1 ml TCEP to 10 ml of 2X Tris-Glycine SDS sample buffer.
2. Add 1 part 2X sample buffer to 1 part sample on ice.
3. Mix well.
4. Heat sample at 95°C - 100°C in a boiling water bath for 4 minutes.
5. Place on ice until ready-to-use or store at –20°C for up to 6 months.

Loading the samples tips for loading samples

— Load the same sample volume in each well

— If the well is not needed for a sample, load with 1X sample buffer

NOTE: If a well is left empty, adjacent samples may spread

- Materials
  - Boiling water bath
  - Ice
- Reagents
  - βME or TCEP
  - 10 ml 2X Tris-Glycine SDS sample buffer

Section X: **Protein Separation in Polyacrylamide Gels**

Loading Buffers — continued

Procedure for sample loading

1. Slowly and gently lift the comb straight up from the gel. Allow air to enter the well area to release the vacuum which forms between the gel and the comb.
2. Rinse each well with 1X electrophoresis buffer.
3. Place the gel into the electrophoresis chamber.
4. Add 1X electrophoresis buffer to cover the wells.
5. Gently load the desired volume of sample beneath the buffer in each well.

NOTE: Loading the sample too fast will lead to diffusion of the sample in the well.

- Materials
  - Electrophoresis chamber
  - Gel
- Reagents
  - Samples
  - 1X Electrophoresis buffer

Section X: **Protein Separation in Polyacrylamide Gels**

Optimal Voltage, Running Times and Power Settings

Optimal voltage

Tris-Glycine polyacrylamide minigels are typically run at constant voltage between 125 - 200 volts. During electrophoresis, the current drops and heat decreases. Voltage set too high, or not limited causes excessive heating, resulting in band distortion and potential damage to the gel and apparatus. Constant voltage allows the same voltage to be used with multiple gels in an apparatus. Gel thickness is not a factor when using constant voltage. For large format gels, a constant current setting with a voltage limit set slightly higher (5 volts) than the expected voltage for the run may also be used to maintain sample velocity.

Optimal electrophoretic time

The gel should be run until the bromophenol blue dye has migrated to the bottom of the gel. Gel running times are dependent upon the buffer system used, the length of the gel and the polyacrylamide concentration. Typically minigels will take approximately 30 - 90 minutes to run. Whereas large format gels may take as long as 5 hours to run.

Section X: **Protein Separation in Polyacrylamide Gels**

ProSieve® 50 Gel Solution

ProSieve® 50 Gel Solution is a modified acrylamide formulation for electrophoresis of large proteins.

See page 60 for ordering information.

Advantages

— **Gradient separation** – From easy-to-cast single concentration gels

— **Easy-to-handle** – Gels are more durable than standard acrylamide

— **Sharp resolution** – Resolves large proteins (>200 kDa)

— **Fast** – Shorter destaining times and faster protein mobility times

— **Low Background** – Even when used with Silver Stain

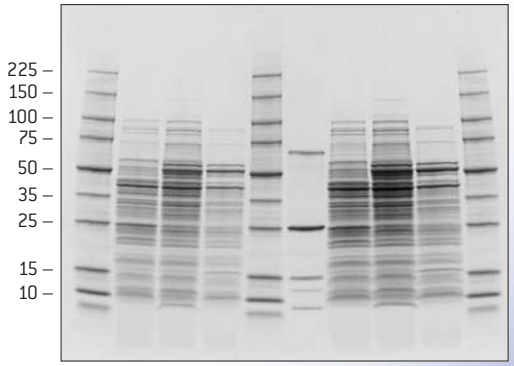
Introduction

PAGEr® Gold Precast Gels are Tris-Glycine (Tris-HCl) gels with a 4% stacking gel. The gels contain no SDS, so can be used for native gel electrophoresis or SDS may be added to the sample and running buffer for denaturing conditions.

Advantages

- Eliminates gel preparation time
- Printed well markings for ease of sample loading
- Easy open cassette
- Compatible with most commonly used vertical mini-gel apparatuses

The photograph below demonstrates the resolution performance of PAGEr® Gold Precast Gels



Separations were run using a 10 well 4-20% Tris-Glycine PAGEr® Gold Precast Gel. The gel was run at 120 volts until the tracking dye reached the base of the gel (approximately 90 minutes). ProSieve® Protein Markers were loaded in lanes 1, 5 and 10; aqueous extracts of three different strains of *E. coli* cells were run in lanes 2 - 4 and 7 - 9. Lanes 2 - 4 were loaded at one half the load level of lanes 7 - 9. Lane 6 contains a mixture of purified proteins. Proteins were detected by Coomassie® Blue Stain.

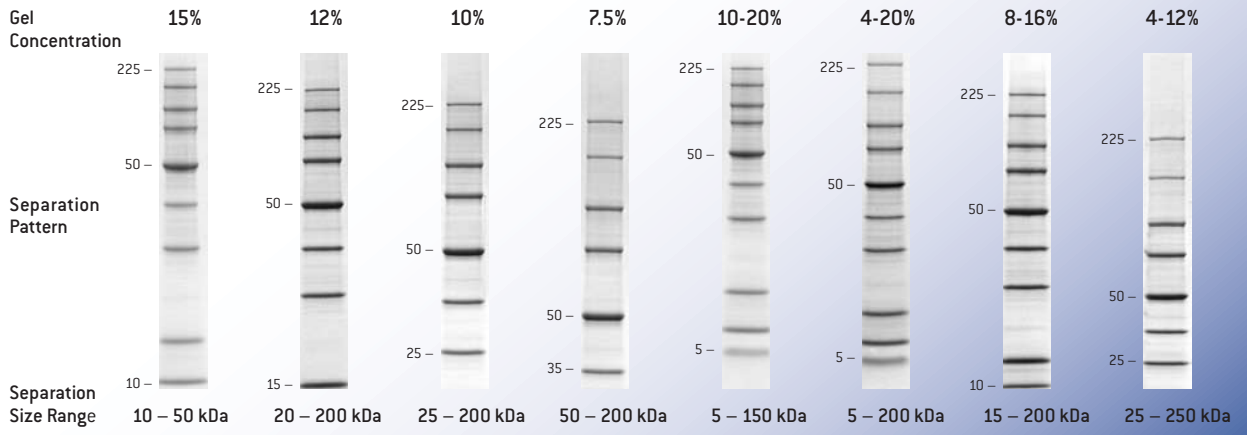
Separation ranges for proteins in PAGEr® Gold Tris-Glycine Gels

Polyacrylamide Concentration	Size Separation Range
7.5%	50 kDa - 200 kDa
10%	25 kDa - 200 kDa
12%	20 kDa - 100 kDa
15%	10 kDa - 50 kDa
4 - 20%	5 kDa - 200 kDa
10 - 20%	5 kDa - 150 kDa
4 - 12%	25 kDa - 250 kDa
8 - 16%	15 kDa - 200 kDa

See page 48 for ordering information.

The figure below demonstrates the separation patterns of ProSieve® Protein Markers on PAGEr® Gold Precast Gels at various concentrations. Smaller molecular weight bands do not separate on some lower concentration gels.

Percentage PAGEr® Precast Gel



ProSieve® Protein Markers contain 10 proteins with exact masses of 225, 150, 100, 75, 50, 35, 25, 15, 10 and 5 kDa. Gels were run at 175 volts until the dye front reached the bottom of the gel (approximately 60 minutes). 8 ml - 10 ml of ProSieve® Protein Marker was loaded per lane (0.8 µg - 1 µg per band). Gels were stained with Coomassie® Blue Stain.

Chamber Compatibility

PAGEr® Gels are available in 9 cm × 10 cm and 10 cm × 10 cm sizes and fit most standard mini-vertical systems.

Contact Scientific Support for information about your specific chamber.

Standard Vertical Systems	PAGEr® Gels
PAGEr® Minigel Chamber	9 cm × 10 cm 10 cm × 10 cm gels
Bio-Rad® MiniPROTEAN® II, MiniPROTEAN® 3 or Ready Gel® Cell Systems Reverse the inner core gasket so the flat side faces outward.	9 cm × 10 cm gels
Novex® XCell SureLock® Mini-Cell Request the spacer for the XCell SureLock® Mini-Cell Chamber from Scientific Support, (Cat. No. 59900).	9 cm × 10 cm 10 cm × 10 cm gels
FisherBiotech® Vertical Minigel FBVE121, Owl Separations Systems Wolverine™ P82 Chamber comes with 2 sets of wedges. Use the thinner wedges for the PAGEr® Gold Gels.	10 cm × 10 cm gels
FisherBiotech® Vertical Minigel FB-VE101, Owl Separations Systems Penguin™ Model P8DS Request adaptor for these chambers from Scientific Support, (Cat. No. 59902).	10 cm × 10 cm gels
Hoefer® Mighty Small (SE250) Replace the buffer chamber with a 'Deep lower buffer chamber for the SE260', order number 80-6148-78, from GE Healthcare.	9 cm × 10 cm 10 cm × 10 cm gels
Daiichi 2, ISS chambers See page 164 for modification instructions.	10 cm × 10 cm gels

Some chambers may require adjustment for optimal fit, see page 164.

Standard Vertical Systems	PAGEr® Gels
Novex® XCell II	9 cm × 10 cm or 10 cm × 10 cm gels
Hoefer® Mighty Small (SE260)	9 cm × 10 cm or 10 cm × 10 cm gels
EC 120 Mini Vertical Gel System	9 cm × 10 cm or 10 cm × 10 cm gels
Biometra® Mini V Chamber	9 cm × 10 cm gels
CBS Scientific MGV System (10 cm × 8 cm units)	9 cm × 10 cm gels
Sigma-Aldrich Mini Techware (11.3 cm × 10 cm units)	10 cm × 10 cm gels
Zaxis System 2000	10 cm × 10 cm gels
Hoefer® Mini VE	10 cm × 10 cm gels

The following guidelines will ensure optimal performance of PAGEr® Gold Precast Gels in these systems.

Bio-Rad® Mini-PROTEAN® II, Mini-PROTEAN® III or Ready Gel® Cell Systems

Remove the rubber gasket from the inner core. Replace the gasket in the reverse orientation into the unit so the flat side faces outward.

Daiichi 2

To run one gel: Place one cassette on wedge side of chamber. Use the taller half of an Owl glass cassette or an equivalent as a buffer dam on the other side. Use Daiichi wedges. The PAGEr® Gold Cassettes cannot be used as the dam in this system.

To run two gels: Widen the hole on the yellow port of the inner core. Replace the long arm wedges with modified wedges, which are thicker and shorter. This chamber modification and the new wedges are available from Lonza free of charge. Contact Lonza Scientific Support for details.

FisherBioTech® Vertical Minigel Protein System: FB-VE10-1 Mini Chamber

Lonza offers an adapator for this chamber, contact Lonza Scientific Support for details. The adapter only works if the inner gasket is white.

Replace black-plastic side spacer with Lonza adapator. Use one on each side of the inner core.

FisherBioTech® Vertical Minigel Protein System: FB-VE12-1

Chamber comes with 2 sets of wedges. Use the thinner wedges with PAGEr® Gold Precast Gels.

Hoefer® Mighty Small (SE250)

Replace the lower buffer chamber with a Deep Lower Buffer Chamber for the SE260. Available from GE Healthcare (Part No. 80-6148-78). The extra depth of the SE260 buffer chamber allows the lid to lock into place.

Novex® XCell SureLock® Mini-Cell

Lonza offers a spacer for this chamber, contact Lonza Scientific Support for details.

To run one gel: Put the gel in the front of the chamber. Put the buffer dam on the back. Place the Lonza spacer between the buffer dam and the buffer core, on the side of the chamber with the gel tension wedge. Lock the gel tension wedge.

To run two gels: Put a gel on each side of the buffer core. Place the Lonza spacer between the gel and the buffer core, on the side of the chamber with the gel tension wedge. Lock the gel tension wedge.

Owl Separation Systems Penguin™ Model P8DS-1

Lonza offers an adaptor for this chamber, contact Lonza Scientific Support for details. The Lonza adaptor for the Penguin™ model only works if the inner gasket is white. Replace black-plastic side spacer with Lonza adaptor. Use one on each side of the inner core.

Procedure for electrophoresis using PAGEr® Gold Precast Gels

1. Cut open pouch and remove gel.
2. Rinse the gel with distilled or deionized water.
3. Slowly and gently lift the comb straight up.

NOTE: Put the comb aside so it can be used to separate the cassette plates at the end of the run.

4. Remove white tape from bottom of the cassette.

NOTE: The sharp end of the comb can be used to peel off the tape.

5. Mount the cassette(s) into the electrophoresis chamber so the printed side faces the outer (anode) buffer chamber. If running only one gel, mount the appropriate buffer dam.
6. Fill the buffer chambers with 1X running buffer.
7. Wash the wells with 1X running buffer, displacing any air bubbles in the wells.
8. Load samples.

NOTE: For best results, load 1X sample buffer in the wells without samples.

9. Run the gels at a constant voltage of 125 - 200 volts until the dye front is near the bottom of the gel (approximately 30 - 90 minutes).
10. Remove the gel(s) from electrophoresis chamber.
11. Place the cassette on a flat surface with the short side of the cassette facing up.
12. Using the end of the comb and starting at the top of the cassette separate the two plates by using a twisting motion to crack the cassette.
13. Carefully remove the short plate.
14. Hold the plate with the gel over an open container.

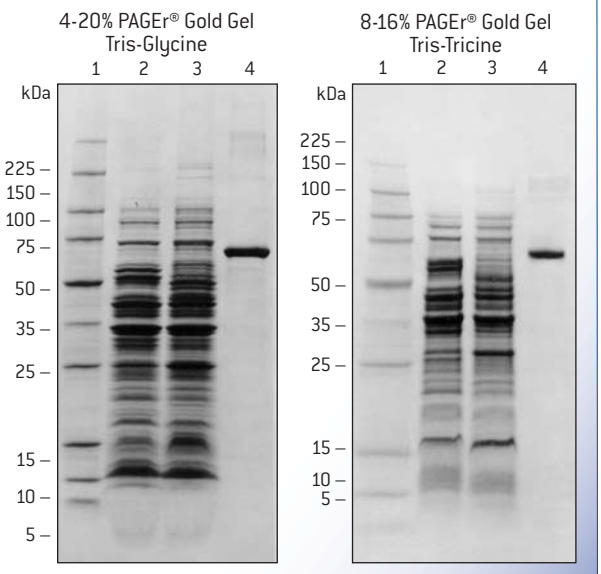
If the gel is adhered to	Then
Large Plate	Run end of comb or a gloved finger behind slot on cassette to push out the lip at the bottom of the gel.
Small Plate	Use the comb or a spatula to loosen one lower corner of the gel.

15. Allow gel to peel away and gently drop into the container.
16. Fix, stain and destain or blot the gel as desired.

- Materials
  - Scissors
  - Pipette
  - Electrophoresis apparatus
  - Power Supply
  - Container
  - Spatula
- Reagents
  - Distilled water
  - Running Buffer AccuGENE® 10X Tris-Glycine or Tris-Glycine SDS Buffer

PAGEr® Gold Precast Gels can be run at higher voltages to achieve faster run times. If using Tris-glycine Running Buffer gels can be run at 200 - 250 volts for approximately 40 minutes. If using Tris-Tricine running buffer gels can be run at 150 - 200 volts for approximately 30 minutes.

The photographs below show the separation of protein samples on PAGEr® Gold Precast Gels under increased voltage.



Samples were prepared using Tris-Glycine SDS Sample Buffer and β-Mercaptoethanol as a reducing agent. Lane 1: ProSieve® Protein Markers; Lanes 2&3: *E. coli* extracts; Lane 4: Bovine Serum Albumin (BSA). Gel 1: 4-20% gel in 1X Tris-Glycine SDS Buffer run at 230 volts for 40 minutes. Gel 2: 8-16% gel in 1X Tris-Tricine SDS Buffer run at 185 volts for 30 minutes. Samples were run until the tracking dye reached the base of the gels. Proteins were detected using Coomassie® Blue Stain.

Detecting proteins with SYPRO® Protein Gel Stains

SYPRO® Protein Gel Stains are highly sensitive fluorescent stains for the rapid detection of proteins in polyacrylamide gels. These stains can detect as little as 4 ng - 8 ng of protein per band in 40 - 60 minutes without destaining. Gels stained with SYPRO® Protein Gel Stains exhibit low background and minimal protein-to-protein staining variability. Gels can be documented using standard imaging systems.

Tips for staining gels with SYPRO® Protein Gel Stains

— The SDS front at the bottom of the gel stains heavily with SYPRO® Stains. Unless the protein of interest comigrates with the SDS front, we recommend running the SDS front off the end of the gel

— Colored stains such as Coomassie® Blue Stain and colored protein markers may interfere with SYPRO® staining and quench fluorescence. To stain gels previously stained with Coomassie® Blue Stain, soak the gel in several changes of 7.5% acetic acid to remove the Coomassie® Stain. Then incubate the gel in 0.05% SDS for 30 minutes and stain with SYPRO® Stain as usual

— Glove powder can leave background markings on gels. Rinse or wash gloves prior to handling gels

— Clean the surface of the transilluminator after each use with deionized water and a soft cloth. Fluorescent stains (such as SYPRO® Stains or ethidium bromide) can accumulate on the transilluminator and may cause high background

— Handle gels carefully to avoid non-specific staining of areas of the gel that have been squeezed

— SYPRO® Stains may be photobleached after several minutes of exposure to UV light. If a gel becomes photobleached, restain by incubating in the staining solution

— SYPRO® stained gels can be restained with Coomassie® Blue or Silver Stain procedures

— Plastic wraps and GelBond® Film will autofluoresce when exposed to UV light resulting in very high background. Gels backed with GelBond® Film can be photographed by inverting the gel on the transilluminator

Select the Best Stain for your Application

Application	SYPRO® Ruby	SYPRO® Tangerine	SYPRO® Red
High performance staining	■		■
Staining prior to Western blotting		■	
2D electrophoresis	■		
Edman microsequencing	■	■	■
Mass spectrometry	■	■	■
Quantitation	■	■	■
Zymography	■	■	■
Electroelution	■	■	■
Membrane staining	■		
Protein expression	■		
Detection prior to Immunostaining	■	■	
Difficult to stain proteins	■		
IEF Gels	■		

- Materials
  - Clear polypropylene container (e.g., Rubbermaid® Recycling #5 Plastics)
- Reagents
  - 7.5% (v/v) acetic acid
  - SYPRO® Protein Gel Stain stock solution

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

Caution: Acetic acid causes burns and respiratory irritation. Take precautions to prevent exposure.

General Procedure for staining proteins with SYPRO® Protein Gel Stains

1. Run SDS-polyacrylamide gels according to standard protocols.

NOTE: To reduce background staining with SYPRO® Red and Tangerine Stains, use 0.05% SDS in the running buffer. Gels run in 0.05% SDS show no change in the migration pattern of proteins.

2. Dilute the 5,000X concentrate to a 1X solution, in 7.5% (v/v) acetic acid, in a clear plastic polypropylene container. For example, for every 10 ml acetic acid add 2 µl stock SYPRO Stain solution.
3. Mix well.
4. Place the gel into the staining container and cover with a lid to protect from light.
5. Gently agitate the gel at room temperature.
6. Stain the gel for 40 - 60 minutes.

NOTE: The optimal staining time depends on the thickness of the gel and the gel concentration. Longer staining times may be required as gel thickness increases.

7. Briefly rinse the gel in 7.5% acetic acid.
8. Remove the gel from the staining container and photograph the gel following the procedure on page 168.

Procedure for staining nondenaturing gels with SYPRO® Red Protein Gel Stain

Options for staining proteins with SYPRO® Red Stain after native gel electrophoresis:

1. Dissolve the stain 1:5,000 in distilled water and follow the general protocol for staining proteins with SYPRO® Protein Gel Stains. This will be highly protein-selective, and will be less sensitive than staining proteins in SDS gels. To increase the signal, a long film exposure can be used since the background fluorescence is essentially zero.
2. Soak the gel after electrophoresis in 0.05% SDS for 30 minutes, then stain with a 1:5,000 solution of SYPRO® Stain diluted in 7.5% acetic acid. Proteins will be denatured and fixed after electrophoresis using this treatment.

Procedure for staining proteins with SYPRO® Tangerine Protein Gel Stain: Non-fixing protocol

SYPRO® Tangerine Protein Gel Stain is an extremely versatile fluorescent stain that does not alter protein structure, interfere with the transfer of proteins to blotting membranes, or use organic solvents. The stain can be diluted into a wide range of buffers with a pH range from 4 - 10 and if fixing is necessary, it can be diluted into 7.5% acetic acid following the General Protocol for Staining Proteins with SYPRO® Gel Stains.

If proteins are to be used for subsequent analysis, dilute SYPRO® Tangerine stock solution into 50 mM phosphate, 150 mM NaCl, pH 7.0 or use one of the buffers listed below prepared as 50 mM - 100 mM solutions containing 150 mM NaCl.

Compatible Buffers	
Formate pH 4.0	HEPES pH 7.5
Citrate pH 4.5	Tris acetate pH 8.0
Acetate pH 5.0	Tris-HCl pH 8.5
MES pH 6.0	Tris borate, 20 mM EDTA pH 9.0
Imidazole pH 7.0	Bicarbonate, pH 10.0

- Materials
  - Clear polypropylene container (e.g., Rubbermaid® Recycling #5 Plastics)
- Reagents
  - 50 mM Phosphate, 150 mM NaCl, pH 7.0 or suitable buffer

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

Follow this procedure if the gel will be used for subsequent analysis such as zymography or Western blotting. Stained proteins can also be eluted from gels and used for further analysis such as mass spectrometry.

1. Run SDS-polyacrylamide gels according to standard protocols. It is not necessary to decrease the amount of SDS present in the running buffer when using SYPRO® Tangerine Gel Stain.
2. Dilute the 5,000X concentrate to a 1X solution, in 7.5% (v/v) acetic acid solution, in a clear plastic polypropylene container. For example, for every 10 ml acetic acid add 2 µl stock SYPRO® Stain solution.
3. Mix well.
4. Place the gel into the staining container and cover to protect from light.
5. Gently agitate the gel at room temperature.
6. Stain the gel for 40 - 60 minutes. The optimal staining time depends on the thickness of the gel and the gel concentration. Longer staining times may be required as gel thickness increases.
7. Remove the gel from the staining container and immediately photograph the gel following the procedure on page 168.

NOTE: If the gel will be blotted after staining with SYPRO® Tangerine Stain, stain the gel according to the procedure above, using 50 mM phosphate, 150 mM NaCl as the stain diluant. Add 0.1% SDS to the transfer buffer. This will help in the transfer of some proteins to the membrane.

Viewing gels stained with SYPRO® Protein Gel Stains

All SYPRO® Protein Gel Stains have two excitation wavelengths; in the UV region at approximately 300 nm and in the blue range of visible spectrum, between 470 and 610 nm. The stains can be visualized using a UV transilluminator or the Dark Reader® Transilluminator.

Photographing gels stained with SYPRO® Protein Gel Stains

— Protein bands stained with SYPRO® Protein Gel Stains are best seen by photographing the gel. The integrating effect of a camera or imaging system can detect bands that are not visible to the eye.

— Gels stained with SYPRO® Protein Gel Stains can be photographed using Polaroid® Cameras, CCD camera systems or laser scanners.

— The highest sensitivity using a Polaroid® Camera can be obtained using Polaroid® 667 black-and-white print film and the SYPRO® Protein Gel Stain Photographic Filter. Exposure time will vary with the intensity of the illumination source. Begin with an f-stop of 4.5 and an exposure of 2 - 8 seconds. Use of an ethidium bromide filter is not recommended as it blocks much of the light and leads to lower detection sensitivity

— The SYPRO® Photographic Filter does not work with CCD Camera systems. For CCD cameras, use the emission and excitation data below and check with the camera manufacturer for the appropriate filter

Stain	Emission (nm)	Excitation (nm)
SYPRO® Red	630	300 and 550
SYPRO® Orange	570	300 and 470
SYPRO® Tangerine	640	300 and 490

Detecting proteins with Coomassie® Blue Stain

Coomassie® Blue Stain binds nonspecifically to most proteins. Proteins are fixed and stained in a Coomassie® Blue staining solution and subsequently destained to eliminate the blue background from the gel. Gels can be dried, photographed or stored wet.

Coomassie® Blue Stain solution	
1X Working Solution	Amount for 1X working Solution*
40% Ethanol	400 ml Ethanol
0.125% Coomassie® Blue	1.25 g Coomassie® Blue R-250
Distilled water	500 ml Distilled water
10% Acetic acid	100 ml Acetic acid

*Add reagents in the order provided

Coomassie® Blue destain solution	
1X Working Solution	Amount for 1X working Solution*
5% Ethanol	50 ml Ethanol
7.5% Acetic acid	75 ml Acetic acid
Adjust volume to 1 liter with distilled water	

Caution: These solutions should be prepared under the fume hood.

Procedure

1. Prepare enough Coomassie® Blue Staining Solution to cover the surface of the polyacrylamide gel.
2. Prewarm the Coomassie® Blue Stain Solution to 55°C.
3. Remove the gel from the glass plates.
4. Submerge the gel in the Coomassie® Blue Stain Solution.
5. Gently agitate the gel for 20 minutes at 55°C until the gel becomes blue. Alternatively, solutions may be heated to 45°C and incubated at room temperature.
6. Decant stain solution from container.

NOTE: Coomassie® Blue Staining Solutions can be saved and stored to stain multiple gels. As the stain solution reaches its "use limit", gels will appear grainy and will not destain completely and new staining solutions should be prepared.

7. Briefly rinse excess stain from gel in water
8. Transfer the gel into the destain solution.
9. Gently agitate the gel at 55°C until the gel has destained and bands are visible. (Approximately 1 hour).

NOTE: The destain solution may need to be changed occasionally until the background is clear. Do not over destain, which can lead to loss of band intensity. Pieces of paper towel or a Kimwipe® can be placed in the corner of the container to speed up this process. Change the tissues when they are saturated with Coomassie® Blue Stain.

- Materials
  - Kimwipes or paper towels
  - 3 plastic containers with covers
  - Shaking incubator set to 55°C
- Reagents
  - Stain solution
    - 0.125% Coomassie® Blue, 40% Ethanol, 10% Acetic acid
  - Destain solution
    - 5% Ethanol, 7.5% Acetic acid

Detecting proteins with silver stain

Silver staining is based on differential reduction of silver ions bound to sulfhydryl and carboxyl side chains on proteins. After electrophoresis, proteins are fixed, exposed to silver nitrite and developed to form a black precipitate of silver. All silver staining procedures are time consuming. Many good kits are commercially available which make the procedure faster and easier. The BioRad® Silver Stain Plus™ Kit is recommended for its versatility and ease of use.

The procedure described below is a modification of Morrisey. This procedure uses dithiothreitol (DTT) to improve reproducibility. An advantage of this version is that development occurs more slowly than many silver staining protocols, giving more control over the final image.

Procedure

Wear gloves and use only glass-distilled water and glass staining containers. All steps can be done at room temperature.

1. Gently agitate the gel in 100 ml Destain I for 30 minutes to overnight.
2. Remove Destain solution I.
3. Gently agitate the gel in 100 ml of Destain II for 30 minutes.
4. Remove Destain II.
5. Gently agitate the gel in 100 ml cross-linking solution for 30 minutes.

NOTE: For small peptides, incubate with glutaraldehyde overnight to insure retention of the peptides in the gel.

6. Remove cross-linking solution.
7. Wash gel with several changes of water over a 2 hour period.

NOTE: Alternatively, the gel can be placed in 2 liters of water and the next morning washed for 30 minutes in fresh water. High background will result if the glutaraldehyde is not completely washed out of the gel.

8. Gently agitate the gel in DTT solution for 30 minutes.
9. Remove DTT solution and drain well, but do not rinse the gel.
10. Gently agitate the gel in 100 ml silver nitrate solution for 30 minutes.

Continued on next page.

11. Place the staining tray under running deionized water and swirl for a few seconds.
12. Remove the water.
13. Add 50 ml of Developing solution, swirl briefly, then discard the solution.
14. Repeat step 13.

NOTE: This reacts with excess silver and prevents non-specific staining of the gel.

15. Add 100 ml of Developing solution and shake slowly.

NOTE: Staining occurs slowly at first but then progresses rapidly. Development takes approximately 5 to 10 minutes.

16. When the bands are slightly lighter than the desired staining level, remove developing solution, rinse quickly with water, add Destain II to cover the gel, as the stop solution. Alternatively, add 5 ml of Stop Solution to the developer to stop development. In either case, development will not stop immediately but continues for approximately 5 minutes after adding Stop Solution.
17. Wash the gel several times in Destain II.
18. Rinse the gel with water.
19. Store in water or dry the gel.

■ Materials

- Glass containers
- Orbital shaker

■ Reagents

- Glass distilled water
- Destain I (40% Methanol 7% Acetic acid)
  - 400 ml Methanol
  - 70 ml Acetic acid
  - Adjust volume to 1 liter with distilled water
  - Store at room temperature indefinitely
- Destain II (5% Methanol, 7% Acetic acid)
  - 700 ml Acetic acid
  - 500 ml Methanol
  - Adjust volume to 10 liters with distilled water
  - Store at room temperature indefinitely
  - Cross-linking solution (10% glutaraldehyde)
  - 20 ml of 50% glutaraldehyde stock in 100 ml of distilled water
- Dithiothreitol (DTT) Solution (5 mg/ml)
  - 5.0 mg DTT in 1 liter of distilled water
- Silver Nitrate Solution (0.1% w/v Silver nitrate)

Caution: Glutaraldehyde is toxic and must be handled in a fume hood

  - 1.0 g Silver nitrate ($AgNO_3$) in 1 liter of distilled water
- 3% Sodium Carbonate (3% w/v)
  - 60.0 g Sodium carbonate in 2 liters of distilled water
  - Store in glass container
- Developing Solution (3% Sodium carbonate, 0.019% Formaldehyde)
  - 200 ml of 3% Sodium carbonate
  - 100 ml of 37% Formaldehyde
  - Prepare just prior to use
- Stop Solution (2.3 M Sodium citrate)
  - 67.64 g Sodium citrate, dihydrate (FW 294.1)
  - Adjust volume to 100 ml in distilled water

Caution: Materials and methods shown here present hazards to the user and the environment. Refer to the safety information on page 210 before beginning these procedures.

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