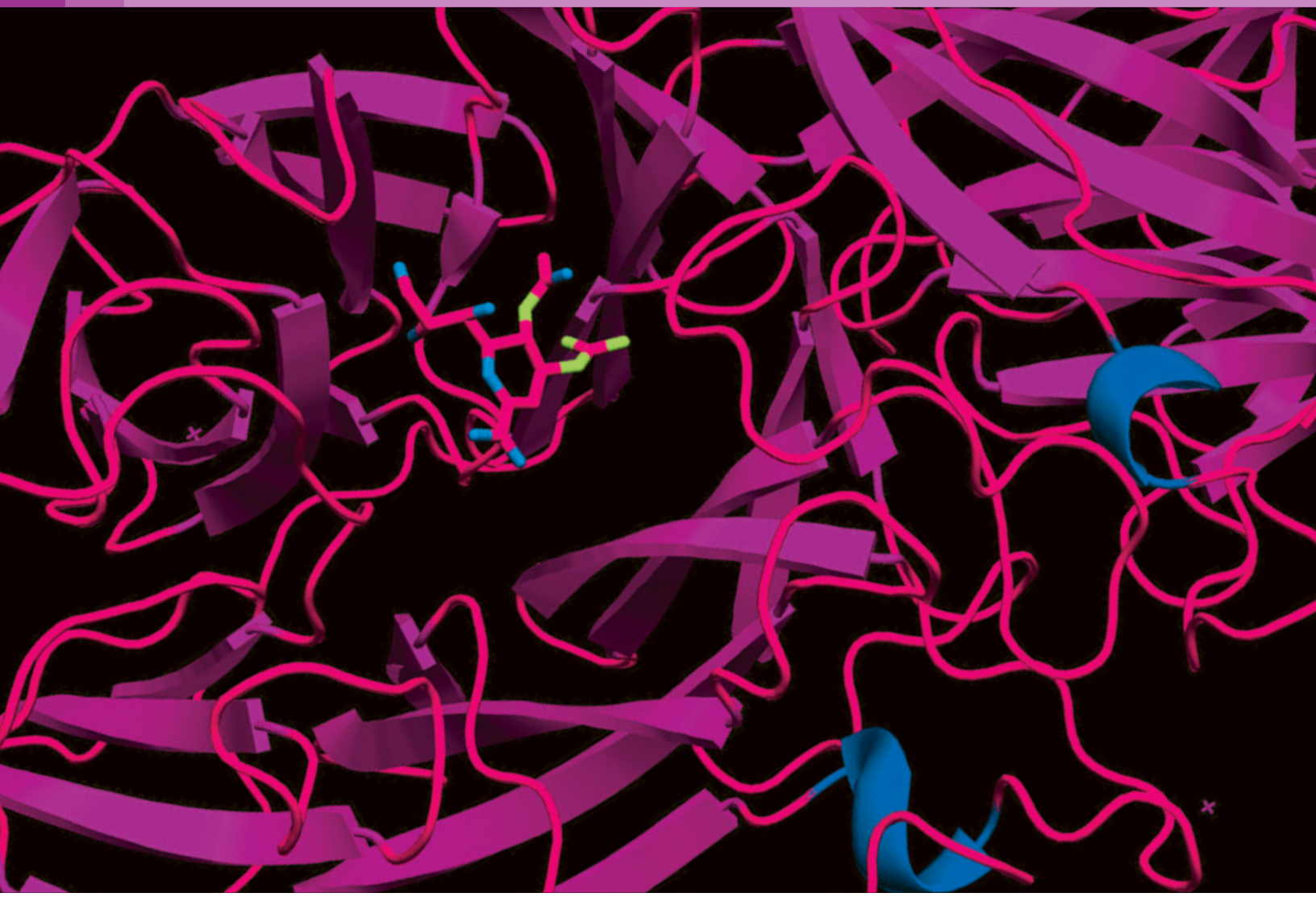
Protein Electrophoresis

EZ-Run Protein Gel Solution | EZ-Run Protein Standards
EZ-Run Gel Staining Solution | Traditional SDS-PAGE Reagents



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Introduction

Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) is the most direct method for assessing the relative molecular weight (MW) of denatured polypeptide chains and the purity of a protein preparation. In SDS-PAGE, the sample gel is first treated with the anionic detergent sodium dodecyl sulfate, which denatures the proteins in the sample and binds tightly to the protein molecules. The SDS molecules confer a negative charge to the polypeptide in proportion to its length. When an electric current is applied across the gel, proteins migrate through the gel matrix toward the anode. In this way, SDS-PAGE separates proteins according to size since the SDS-coated proteins have a uniform charge: mass ratio. Proteins with lower molecular weight (MW) travel more quickly through the gel than those with larger mass because of the sieving effect of the gel matrix.

Shapiro et al. *Biochem. Biophys. Res.* **28**, 815	Laemmli. *Nature.* **277**, 680.	Lambin. *Anal. Biochem.* **85**, 114.	Schagger & von Jagow. *Anal. Biochem.* **166**, 368.	Thermo Fisher Scientific, Fisher BioReagents
1967	**1970**	**1978**	**1987**	**2015**
SDS-PAGE for separating proteins by molecular weight	**Discontinuous SDS-PAGE** for greater resolution	**Gradient Gels** for wider separation range	**Tricine-SDS-PAGE** for separating small proteins	**FASTRun™ Tris SDS PAGE Running Buffer** for speed, clarity and resolution on gels sets

Table 1. Advances in SDS-PAGE for Characterization of Proteins

The SDS-PAGE technique has been refined over the years (Table 1). For example, specialized gel systems, such as porosity gradient gels and Tricine-SDS-PAGE, were developed to expand the MW analysis range and to improve the resolution of small proteins, respectively. Many would agree that improvements to the technique have reached a plateau, and standard protocols have been adopted in most laboratories around the world.

Now, thanks to novel buffer chemistry, **there is a faster way to separate protein gels**.

FASTRun™ Tris SDS PAGE Buffer

For years, chemists have been relying on traditional Tris SDS PAGE running buffers used with tris glycine laemmli gels to separate proteins, waiting an average of 40 minutes per gel. Fisher BioReagents developed FASTRun Tris SDS PAGE Running Buffer, 10X, to cut the time you spend waiting for your gels to finish. In a fraction of the time, FASTRun buffer delivers clear separation of protein bands while you are still waiting for the traditional TG running buffer. The clarity is amazing even at a wide range of molecular weights. View our video at **www.fishersci.com/FastRUNSDSBuffer** to see it yourself.

Fisher BioReagents FASTRun Tris SDS PAGE Running Buffer, 10X, is designed to be used with economical and readily available Tris-Glycine SDS chemistry whether you buy premade or pour your own (PYO) polyacrylamide gels.

- Uses novel buffer chemistry to give you better resolution, requiring fewer TG gel compositions to fully resolve a protein
- Saves time, freeing you to do the things that matter to you, be more productive and perform more electrophoresis runs per day
- Compatible with standard polyacrylamide gels, both premade and pour your own
- Compatible with all commercially available protein electrophoresis tanks

Buffer System for Tris-Glycine Gels	Voltage (Constant)	Typical Current	Final Buffer Temperature	Run Time
10X Tris-Glycine SDS Running Buffer	125V	Start: 30–40 mA End: 8–12 mA	+24 to +28°C	90 minutes (dependent on gel percentage)
FASTRun™ Tris SDS PAGE Running Buffer, 10X	200V*	Start: 95–105 mA End: 52–56 mA	+32 to +38°C	22 minutes (dependent on gel percentage and voltage)

* Voltages of up to 250V may be used to reduce run time. We recommend running your gels at 200V. For more information about voltage, resolution and separation, visit: **www.fishersci.com/FastRUNSDSBuffer**.

Catalog No.	Description	Quantity
BP881-500	Tris SDS PAGE Running Buffer, 10X	500mL
BP881-1	Tris SDS PAGE Running Buffer, 10X	1L

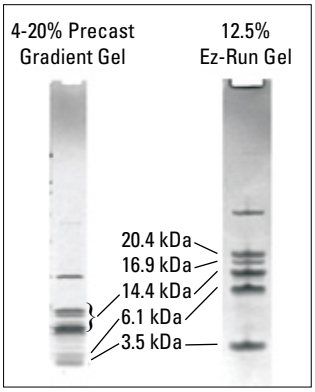
EZ-Run Protein Gel Solution

- Ready to use, proprietary gel chemistry
- Superior resolution with wide separation range on same mini-gel
- No stacking gel required
- Stable for two years at room temperature
- Compatible with all conventional staining methods
- Suitable for post-electrophoresis applications such as Western blot transfer and MALDI analysis

EZ-Run Protein Gel Solution is a unique ready-to-pour premixed solution of acrylamide, buffer and SDS that enables superior resolution of protein bands by SDS-PAGE. The liquid blend requires only the addition of ammonium persulfate and TEMED to prepare a quality gel matrix for SDS-PAGE. The proprietary gel chemistry imparts gradient-like properties to the polymerized gel matrix, enabling the separation of small peptides and high molecular weight proteins on the same mini-gel.

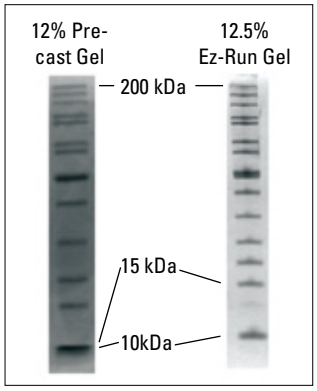
EZ-Run gel matrix is used as a simple, continuous gel system and does not require a stacking gel, which saves labor and time in casting the gel. EZ-Run gel separates small proteins like Tricine-SDS-PAGE and has a wide separation range similar to gradient gels (3 to 200kDa on the same mini-gel).

EZ-Run gels are compatible with all standard electrophoresis equipment, as well as common staining methods such as Coomassie blue, silver stain and fluorescent dyes. Post-electrophoresis techniques such as Western blot transfer, protein sequencing and MALDI analysis can also be applied to proteins separated on EZ-Run gels.



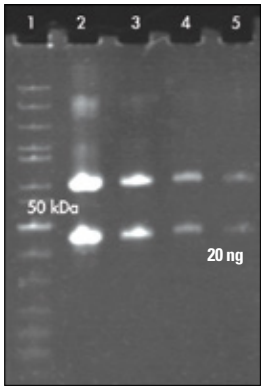
Resolution equal to or better than gradient precast gels!

EZ-Run Protein Gel Solution provides superior separation of closely spaced, small proteins (<20kDa) compared to a commercial gradient precast gel.



Separate wide range of protein sizes (3–200kDa) on the same minigel.

The EZ-Run continuous gel system enables separation of small peptides and high MW proteins on the same minigel. For example, a commercial 12% precast discontinuous gel is not capable of resolving the 10 and 15kDa proteins compared to the 12% EZ-Run gel.



EZ-Run gel matrix is compatible with common gel staining methods, such as fluorescent dyes.

Serial dilution of BSA (66kDa) and Ovalbumin (45kDa) are loaded in lanes 2 to 5 of an EZ-Run gel and detected with SYPRO™ Ruby fluorescent protein stain. Protein standard in lane one is BP3602 EZ-Run Rec Protein Ladder.

EZ-Run Protein Gel Solution Separation Range:

EZ-Run Gel Percent	MW Separation Range (kDa)
10	10–220
12.5	3–200
15	2–100

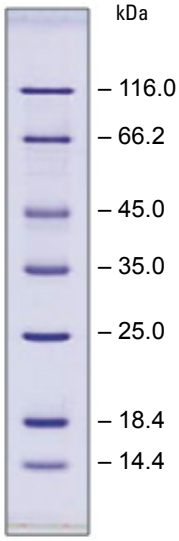
EZ-Run Protein Gel Solution Ordering Information:

Description	Size	Catalog No.
10% EZ-Run Protein Gel Solution with Buffer	500mL	**BP7710-500**
12.5% EZ-Run Protein Gel Solution with Buffer	500mL	**BP7712-500**
15% EZ-Run Protein Gel Solution with Buffer	500mL	**BP7715-500**
20x Running Buffer for EZ-Run Protein Gel Solution	500mL	**BP7700-500**

EZ-Run Protein Standards

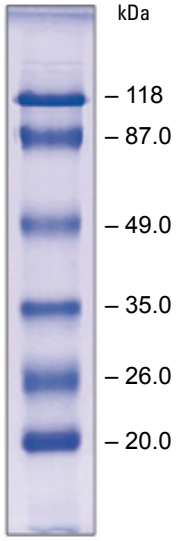
Designed to assist in characterizing unknown proteins in polyacrylamide gels and immunoblots

- Highly purified markers and ladders provide compact and clear bands
- Prestained standards are indispensable in monitoring protein separation and transfer efficiency
- Reference bands allow quick gel progress assessment
- Unstained standards are most suitable for precise sizing of proteins
- All standards are supplied in loading buffer and are ready to use



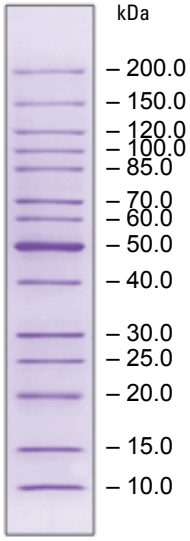
12% SDS-PAGE

EZ-Run Protein Marker
BP3600-500



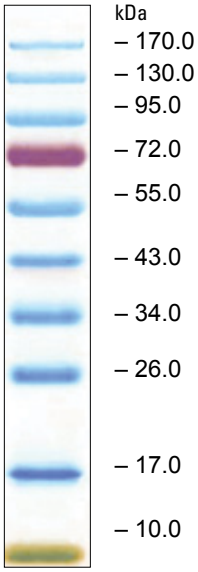
12% SDS-PAGE

EZ-Run Prestained
Protein Marker
BP3601-500 and BP3601-1



4–20% SDS-PAGE

EZ-Run Rec
Protein Ladder
BP3602-500 and BP3602-1



4–20% SDS-PAGE

EZ-Run Prestained
Rec Protein Ladder
BP3603-500 and BP3603-1

Ordering Information MW Range	No. of Bands	Reference Band	Source	Quantity	Catalog No.
Unstained Protein Standards					
14.4-116.0kDa	7	—	Native proteins	500µL	**BP3600-500**
10.0-200.0kDa	14	50kDa	Recombinant proteins	500µL	**BP3602-500**
				2 x 500µL	**BP3602-1**
Prestained Protein Standards					
20.0-118.0kDa	6	—	Native proteins	500µL	**BP3601-500**
				2 x 500µL	**BP3601-1**
11.0-170.0kDa	10	72kDa	Recombinant proteins	500µL	**BP3603-500**
				2 x 500µL	**BP3603-1**

EZ-Run™ Protein Gel Staining Solution

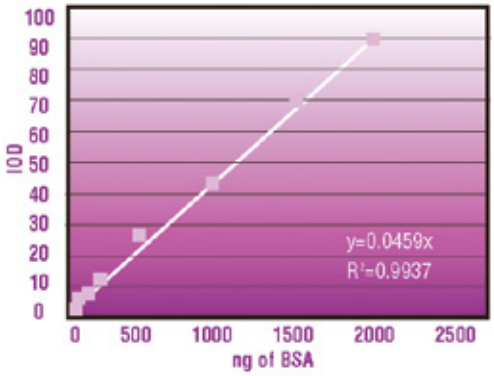
Highly Sensitive, Nontoxic

- Detects as little as 5ng protein
- Produces minimal or no background
- Permits rapid staining/destaining (30-minute staining and one-hour destaining in water; sufficient for most applications)
- Contains Coomassie Brilliant Blue G-250
- Does not contain methanol or acetic acid
- Ready to use

Ordering Information	
Quantity	Catalog No.
1L	BP3620-1
4L	BP3620-4

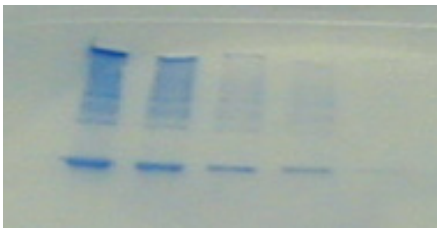
One liter of EZ-Run Protein Gel Staining Solution is sufficient for 50 minigels.

Linear Range of Protein Detection Using BP3620 EZ-Run Protein Gel Staining Solution

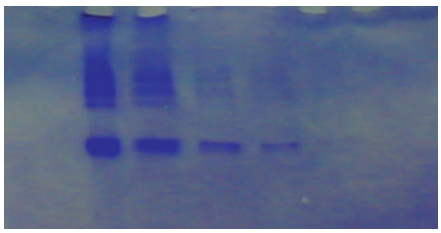


Band intensity was measured and plotted against the amount of protein (BSA) loaded per gel lane. The result shows a linear dynamic range from 5ng to 2,000ng using EZ-Run Protein Gel Staining Solution.

EZ-Run Protein Gel Staining Solution

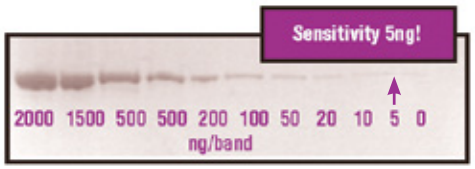


Conventional Coomassie Blue Staining Solution



Destaining of EZ-Run Protein Gel Staining Solution
Compared to conventional Coomassie Blue staining, the EZ-Run stain produces very clean backgrounds using only water for destaining.

Staining Sensitivity with EZ-Run Protein Gel Staining Solution



Serial dilution of BSA on 10% SDS-PAGE demonstrating staining sensitivity of EZ-Run Protein Gel Staining Solution.

Additional Protein Electrophoresis Reagents from Fisher BioReagents

Buffers for Protein Electrophoresis

Description	Quantity	Catalog No.
Protein Gel-Loading Dye for SDS-PAGE		
2x	1mL	**BP637-1**
2x	5mL	**BP637-5**
TG Tris-Glycine		
10X	1L	**BP1306-1**
10X	4L	**BP1306-4**
10X	1L*	**BP1307-1**
TGS Tris-Glycine-SDS		
10X	1L	**BP1341-1**
10X	4L	**BP1341-4**
5X	1L*	**BP1398-92**
10X	1L*	**BP1342-1**
SDS Sodium Dodecyl Sulfate		
10%	200mL	**BP2436-200**
10%	1L	**BP2436-1**
20%	200mL	**BP1311-200**
20%	1L	**BP1311-1**
PBS Phosphate Buffered Saline		
10X	500mL	**BP399-500**
10X	1L	**BP399-1**
10X	4L	**BP399-4**
10X	20L	**BP399-20**
TBS Tris-Buffered Saline		
10X(7.4)	100mL	**BP2471-100**
10X(7.4)	500mL	**BP2471-500**
10X(7.4)	1L	**BP2471-1**

*Pre-weighed powder to make 1L. Dissolves in water.

Detergents/Denaturing Agents

Description	Quantity	Catalog No.
Brij 35	500g	**BP345500**
Chaps	1g	**BP5711**
	5g	**BP5715**
Chapso	500mg	**BP575500**
SDS	100g	**BP166100**
	500g	**BP166500**
	5kg	**BP1665**
SDS, Micropellets	100g	**BP8200100**
	500g	**BP8200500**
	5 kg	**BP82005**
SDS 10% Solution	200mL	**BP2436200**
	1L	**BP24361**
SDS 20% Solution	200mL	**BP1311200**
	1L	**BP13111**
Triton X-100	100mL	**BP151100**
	500mL	**BP151500**
Tween 20	100mL	**BP337100**
	500mL	**BP337500**
Tween 80	500mL	**BP338500**
N-Octl-B-D-Glucpyranoside	1g	**BP5851**
	5g	**BP5855**
	25g	**BP58525**

Additional Protein Electrophoresis Reagents from Fisher BioReagents

Acrylamide, Bis-Acrylamide and Catalysts

Description	Quantity	Catalog No.
Acrylamide	100g	**BP170100**
	500g	**BP170500**
	5kg	**BP1705**
Acrylamide Solution, 40%	1L	**BP14021**
Bis-Acrylamide	25g	**BP17125**
	100g	**BP171100**
Bis-Acrylamide Solution, 2%	250mL	**BP1404250**
Acrylamide:Bis-Acrylamide, Dry Powder Mix, 19:1 (5% Cross-linker)	100g	**BP1364100**
Acrylamide:Bis-Acrylamide, Dry Powder Mix, 29:1 (3.3% Cross-linker)	100g	**BP1366100**
Acrylamide:Bis-Acrylamide, Dry Powder Mix, 37.5:1 (2.6% Cross-linker)	100g	**BP1368100**
Acrylamide:Bis-Acrylamide, 40% Solution, 19:1 (5% Cross-linker)	1L	**BP14061**
Acrylamide:Bis-Acrylamide, 40% Solution, 29:1 (3.3% Cross-linker)	1L	**BP14081**
Acrylamide:Bis-Acrylamide, 40% Solution, 37.5:1 (2.6% Cross-linker)	1L	**BP14101**
Ammonium Persulfate	25g	**BP17925**
	100g	**BP179100**
Sodium Persulfate	1kg	**BP26371**
	130mL (100g)	**BP150100**
TEMED	26mL (20g)	**BP15020**

Protease Inhibitors

Description	Quantity	Catalog No.
4-(2-Aminoethyl)Benzenesulfonyl Fluoride HCL	10mg	**BP264410**
	50mg	**BP264450**
	100mg	**BP2644100**
	500mg	**BP2644500**
	1g	**BP26441**
Aprotinin	1mL	**BP250310**
	40mL	**BP250340**
Benzamidine.HCL	25g	**BP43525**
Leupeptin Hemisulfate	1mg	**BP26621**
	5mg	**BP26625**
	25mg	**BP266225**
	100mg	**BP2662100**
Pepstatin A	5mg	**BP26715**
	10mg	**BP267110**
	25mg	**BP267125**
	100mg	**BP2671100**
	250mg	**BP2671250**

Preparation of Polyacrylamide Stacking and Separating Gels (SDS-PAGE)

Separating Gel (Total Volume, 15mL)[1]

Final Percent Acrylamide in Gel[2] Stock Solutions[3]	5	6	7	7.5	8	9	10	12	13	15
30% Acrylamide/0.8% Bis-Acrylamide	2.50mL	3.00mL	3.50mL	3.75mL	4.00mL	4.50mL	5.00mL	6.00mL	6.50mL	7.50mL
4X Tris•Cl, pH 8.8	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
H_2O[4]	8.6	8.1	7.6	7.35	7.1	6.6	6.1	5.1	4.6	3.6
10% SDS	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
10% Ammonium Persulfate[5]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
TEMED	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Four Percent Stacking Gel (Total Volume, 5mL)[1]

Description	Quantity
Stock Solution	Volume
30% Acrylamide/0.8% Bis-Acrylamide	0.65mL
4X Tris•Cl, pH 6.8	1.25mL
H_2O[4]	3.00mL
10% SDS	50µL
10% Ammonium Persulfate[5]	25µL
TEMED	5µL

Procedure for Gel Preparation

In a 25mL sidearm flask, mix the given volumes of Acrylamide/Bis-Acrylamide solution, Tris-HCl buffer and H_2O. Degas under vacuum 10 to 15 minutes. Add the SDS solution, Ammonium Persulfate solution and TEMED. Swirl gently to mix. Use immediately.

[1] These volumes are adequate for a gel of dimensions 0.75cm x 14cm x 14cm. The recipes are based on the SDS (denaturing)-continuous buffer system of Laemmli (1970).
[2] The % acrylamide selected for the separating gel will depend on the molecular sizes of the proteins being separated.
[3] Recipes for the stock solutions appear earlier in this section.
[4] All reagents and solutions used in this protocol must be prepared with distilled deionized water.
[5] Store at 4°C (maximum five days).

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