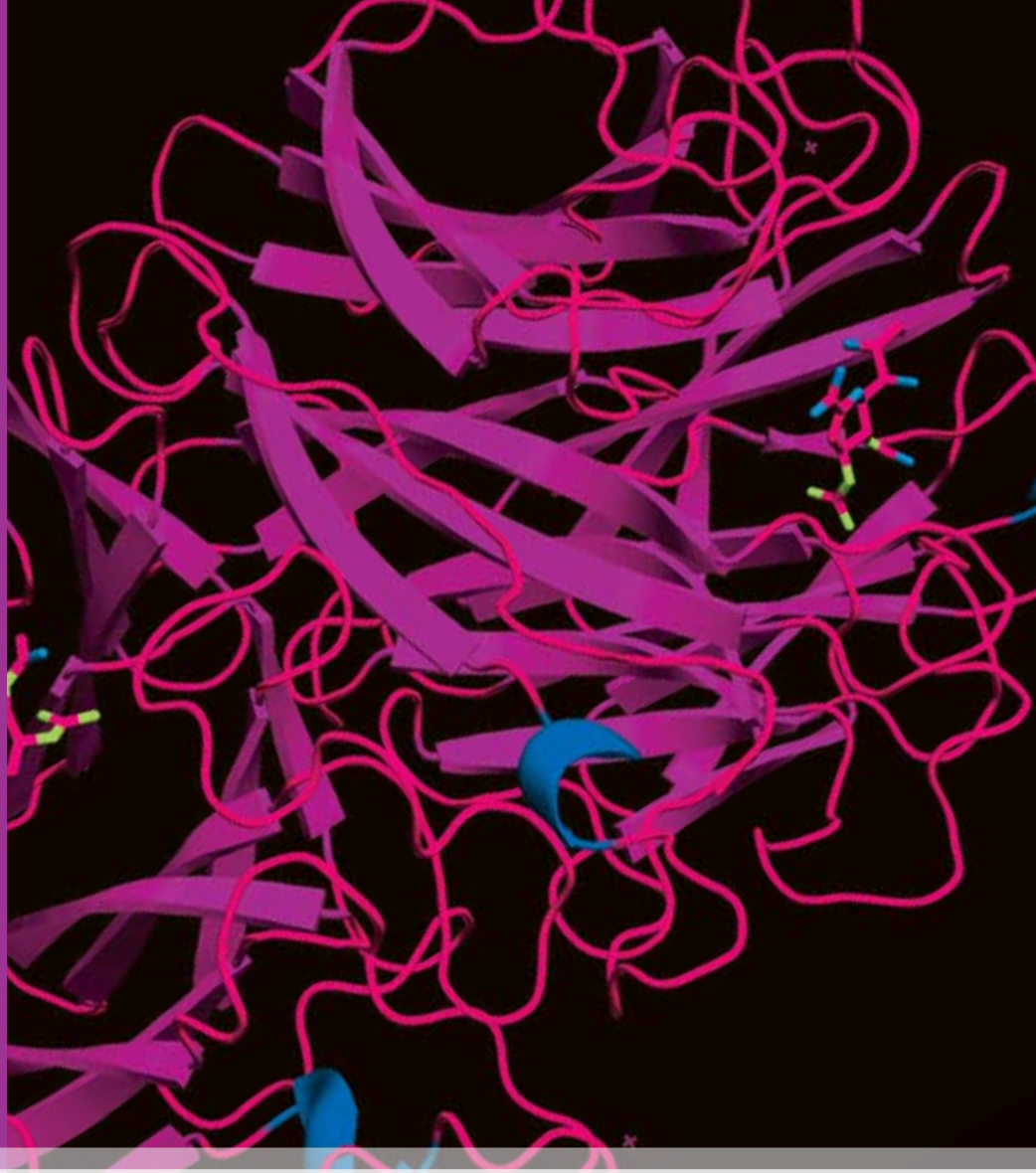
EZ-Run Protein Gel Solution

EZ-Run Protein Standards

EZ-Run Gel Staining Solution

Traditional SDS-PAGE Reagents

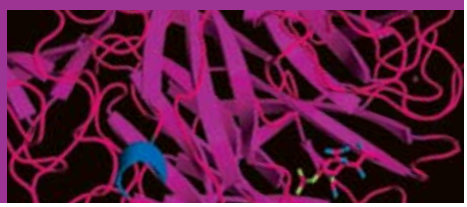


Protein Electrophoresis

Fisher Scientific

Fisher BioReagents®

PROTEIN ELECTROPHORESIS



Introduction

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

Table 1. Advances in SDS-PAGE for characterization of proteins.

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.
1967 SDS-PAGE for separating proteins according to molecular weight	**1970** Discontinuous SDS-PAGE for greater resolution than continuous gel system	**1978** Gradient Gels for wider separation range	**1987** Tricine-SDS-PAGE for separating small proteins	**2009** EZ-Run™ Protein Gel for a high resolution continuous gel system

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 M_r 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.

However, Fisher BioReagents **EZ-Run Protein Gel Solution** is used as a simple, continuous gel system for SDS-PAGE that provides the resolution of a gradient gel with less preparative work than the Laemmli discontinuous gel system (Table 1). It is a premixed solution of acrylamide, bis-acrylamide, buffer, and SDS that eliminates the need of a stacking gel. The gradient-like properties of the EZ-Run gel matrix slow the migration of proteins through the electrophoretic field, enabling the resolution of small peptides and large proteins on the same gel.

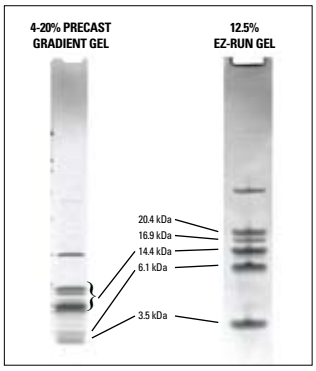
EZ-Run™ Protein Gel Solution

- Ready to use
- Superior resolution
- Wide separation range on same mini-gel
- No stacking gel required
- Proprietary gel chemistry
- 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.

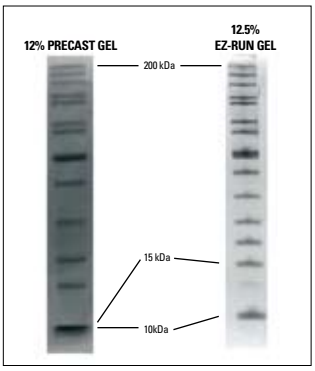
EZ-Run gel matrix is used as a simple, continuous gel system and does not require a stacking gel, which saves labor and time in casting the gel. EZ-Run gel separates small proteins like Tricine-SDS-PAGE and has a wide separation range similar to gradient gels (3 to 200kDa on the same mini-gel).

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.



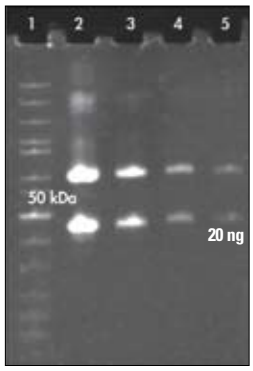
Resolution equal to or better than gradient precast gels!

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 compatible with common gel staining methods such as fluorescent dyes

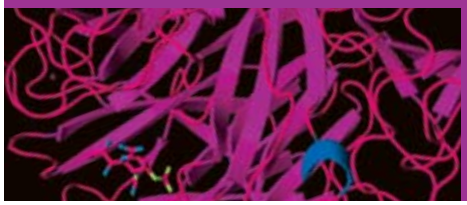
Serial dilution of BSA (66kDa) and Ovalbumin (45kDa) are loaded in lanes 2 to 5 of an EZ-Run gel and detected with SYPRO® Ruby fluorescent protein stain. Protein standard in lane 1 is BP3602 EZ-Run Rec Protein Ladder.

EZ-Run Protein Gel Solution Separation Range:

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

EZ-Run Protein Gel Solution Ordering Information:

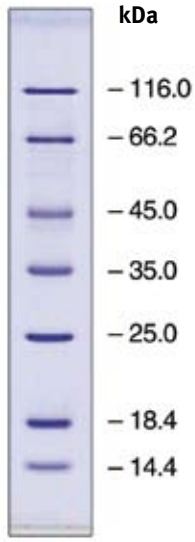
Description	Size	Catalog No.
10% EZ-Run Protein Gel Solution with buffer	100mL	**BP7710-100**
10% EZ-Run Protein Gel Solution with buffer	500mL	**BP7710-500**
12.5% EZ-Run Protein Gel Solution with buffer	100mL	**BP7712-100**
12.5% EZ-Run Protein Gel Solution with buffer	500mL	**BP7712-500**
15% EZ-Run Protein Gel Solution with buffer	100mL	**BP7715-100**
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

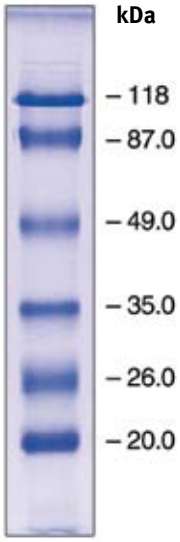
Designed to assist in characterizing unknown proteins in polyacrylamide gels and immunoblots

- 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



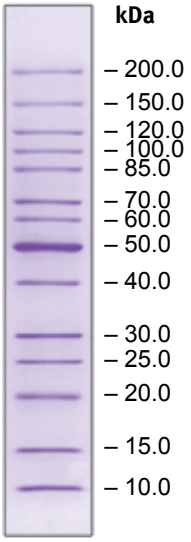
12% SDS-PAGE

EZ-Run Protein Marker
BP3600-500 and BP3600-1



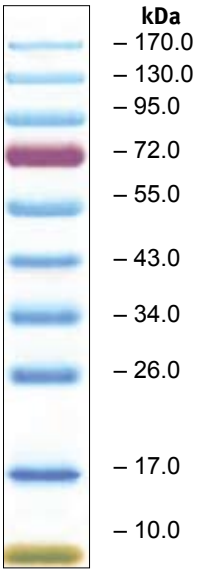
12% SDS-PAGE

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



4-20% SDS-PAGE

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



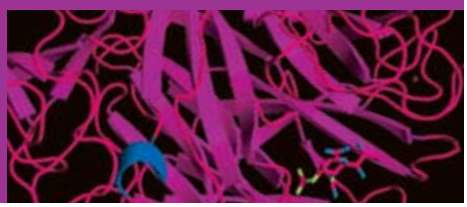
4-20% SDS-PAGE

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

Ordering Information

Description	MW Range	No. of Bands	Reference Band	Source	Quantity	Catalog No.
Unstained Protein Standards	14.4-116.0kDa	7	----	Native proteins	500µL	**BP3600-500**
					2 x 500µL	**BP3600-1**
	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**

PROTEIN ELECTROPHORESIS



EZ-Run Protein Gel Staining Solution

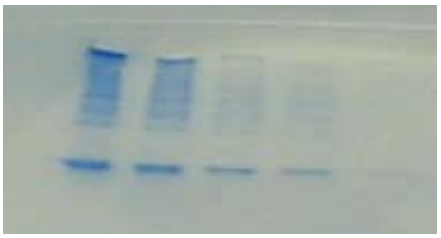
Highly sensitive, nontoxic

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

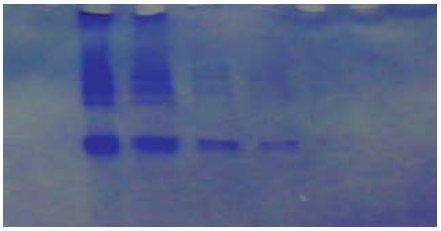
Ordering Information	
Quantity	**Catalog No.**
1L	**BP3620-1**
4L	**BP3620-4**

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

EZ-Run Protein Gel Staining Solution

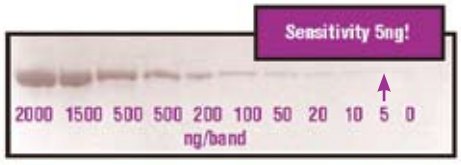


Conventional Coomassie Blue Staining



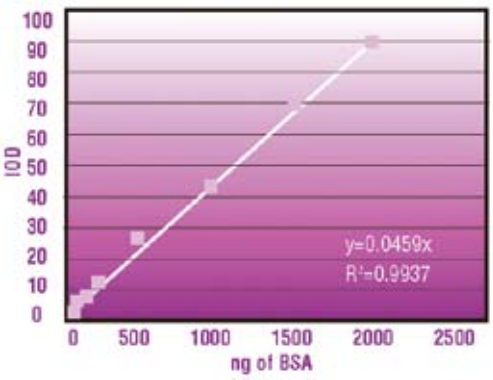
Destaining of EZ-Run Protein Gel Staining Solution
Compared to conventional Coomassie Blue staining, the EZ-Run stain produces very clean backgrounds using only water for destaining.

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.

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 2000ng using 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. Dissolve in water.

Detergents/Denaturing Agents

Description	Quantity	Catalog No.
BRIJ 35	500g	**BP345-500**
CHAPS	1g	**BP571-1**
	5g	**BP571-5**
CHAPSO	500mg	**BP575-500**
SDS	100g	**BP166-100**
	500g	**BP166-500**
	5kg	**BP166-5**
SDS 10% SOLUTION	200mL	**BP2436-200**
	1L	**BP2436-1**
SDS 20% SOLUTION	200mL	**BP1311-200**
	1L	**BP1311-1**
TRITON X-100	100mL	**BP151-100**
	500mL	**BP151-500**
TWEEN 20	100mL	**BP337-100**
	500mL	**BP337-500**
TWEEN 80	500mL	**BP338-500**
N-OCTL-B-D-GLUCPYRANOSIDE	500mg	**BP585-500**
	1g	**BP585-1**
	5g	**BP585-5**
	25g	**BP585-25**

Additional Protein Electrophoresis Reagents from Fisher BioReagents

Acrylamide, Bis-Acrylamide, and Catalysts

Description	Quantity	Catalog No.
Acrylamide	100g	**BP170-100**
	500g	**BP170-500**
	5kg	**BP170-5**
Acrylamide Solution, 40%	1L	**BP1402-1**
Bis-Acrylamide	25g	**BP171-25**
	100g	**BP171-100**
Bis-Acrylamide Solution, 2%	250mL	**BP1404-250**
Acrylamide:Bis-Acrylamide, Dry Powder Mix, 19:1 (5% Cross-linker)	100g	**BP1364-100**
Acrylamide:Bis-Acrylamide, Dry Powder Mix, 29:1 (3.3% Cross-linker)	100g	**BP1366-100**
Acrylamide:Bis-Acrylamide, Dry Powder Mix, 37.5:1 (2.6% Cross-linker)	100g	**BP1368-100**
Acrylamide:Bis-Acrylamide, 40% Solution, 19:1 (5% Cross-linker)	1L	**BP1406-1**
Acrylamide:Bis-Acrylamide, 40% Solution, 29:1 (3.3% Cross-linker)	1L	**BP1408-1**
Acrylamide:Bis-Acrylamide, 40% Solution, 37.5:1 (2.6% Cross-linker)	1L	**BP1410-1**
Ammonium Persulfate	25g	**BP179-25**
	100g	**BP179-100**
Sodium Persulfate	1kg	**BP2637-1 TEMED**
	130mL (100g)	**BP150-100**
TEMED	26mL (20g)	**BP150-20**

Protease Inhibitors

Description	Quantity	Catalog No.
4-(2-AMINOETHYL) BENZENESULFONYL FLUORIDE HCL	10mg	**BP2644-10**
	50mg	**BP2644-50**
	100mg	**BP2644-100**
	500mg	**BP2644-500**
	1g	**BP2644-1**
APROTININ	10mL	**BP2503-10**
	40mL	**BP2503-40**
BENZAMIDINE.HCL	25g	**BP435-25**
	1kg	**BP435-1**
LEUPEPTIN HEMISULFATE	1mg	**BP2662-1**
	5mg	**BP2662-5**
	25mg	**BP2662-25**
	100mg	**BP2662-100**
PEPSTATIN A	5mg	**BP2671-5**
	10mg	**BP2671-10**
	25mg	**BP2671-25**
	100mg	**BP2671-100**
	250mg	**BP2671-250**

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

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

Final % 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]	8.60	8.10	7.60	7.35	7.10	6.60	6.10	5.10	4.60	3.60
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

4% Stacking Gel (Total Volume, 5mL)[1]

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 5 days).

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