



SDS-PAGE Reagents and Protocols

Contents

[Required Reagents and Equipment].....	2
[Electrophoresis].....	2
[Gel Staining].....	3

東京化成工業株式会社

Tel : 03-3668-0489 • 06-6228-1155
Fax : 03-3668-0520 • 06-6228-1158

TOKYO CHEMICAL INDUSTRY CO., LTD.

Tel : +81 (0)3-5640-8878
Fax : +81 (0)3-5640-8902

TCI AMERICA

Tel : 800-423-8616 • 503-283-1681
Fax : 888-520-1075 • 503-283-1987

TCI EUROPE N.V.

Tel : +32 (0)3 735 07 00
Fax : +32 (0)3 735 07 01

梯希爱(上海)化成工业发展有限公司

Tel : 800-988-0390 • 021-67121386
Fax : 021-6712-1385

TCI Chemicals (India) Pvt. Ltd.

Tel : +91 (0)44-2262 0909
Fax : +91 (0)44-2262 8902

SDS-PAGE is an experimental method that uses a polyacrylamide gel to separate proteins based on differences in size. Proteins are initially incubated with the anionic surfactant SDS, causing them to acquire a negative charge proportional to their peptide chain length, which in turn allows them to migrate in approximately the same order as their molecular weight.

[Required Reagents and Equipment]

- Electrophoresis Equipment
- Heat Block
- SDS Sample Buffer

We offer the following four Sample Buffers:

[2X SDS-PAGE Sample Buffer \(2-Mercaptoethanol free\) \[for Electrophoresis\]](#) (TCI Product No. **B5834**)

[4X SDS-PAGE Sample Buffer \(2-Mercaptoethanol free\) \[for Electrophoresis\]](#) (TCI Product No. **B6104**)

[6X Sample Buffer \(2-Mercaptoethanol free\) \[for Electrophoresis\]](#) (TCI Product No. **B6105**)

[2X SDS-PAGE Sample Buffer Phenol Red \(2-Mercaptoethanol free\) \[for Electrophoresis\]](#) (TCI Product No. **B6110**)

*When performing electrophoresis under reducing conditions, addition [2-Mercaptoethanol \[for Electrophoresis\]](#) (TCI Product No. **M1948**) before sample incubation is required.

- SDS Running Buffer (25 mM Tris, 192 mM Glycine, 0.1% SDS)
- Protein Molecular Weight Marker
- Polyacrylamide Gel

Either purchase or prepare an acrylamide gel at the appropriate concentration, using the table below for reference.

Gel Concentration	Molecular weight range that can be fractionated (Da)
7.5%	40,000 - 400,000
10%	20,000 - 300,000
12.5%	10,000 - 250,000
15%	2,000 - 200,000

[Electrophoresis]

1. Combine protein samples with sample buffer and deionized water such that sample buffer reaches a concentration of 1x. Vortex and spin down.
2. Incubate at 100°C for 5 minutes using a heat block then allow to cool to room temperature.
3. Set up electrophoresis equipment with gel, adding running buffer to top and bottom chambers. Clean inside of wells with buffer by pipetting.
4. Apply samples and molecular weight markers to wells. Run at constant current of 20 mA per gel until dye in sample buffer reaches bottom of gel (approximately 60 to 80 minutes).

[Gel Staining]

Dyes:

- [Coomassie Brilliant Blue G-250 \(Ready-to-use solution\) \[for Electrophoresis\]](#) (TCI Product No. **C3488**)
- [Gel Negative Stain kit \[for Electrophoresis\]](#) (TCI Product No. **G0615**)
- [Silver Stain Kit \[for Electrophoresis\]](#) (TCI Product No. **I1309**)

	Dye	Time	Sensitivity	Features
CBB Staining	C3488	2 hours to overnight	Several µg	Simple operation and quantitative.
Negative Staining	G0615	15 to 30 minutes	Several ng	Quick gel staining; decolorized gels can be used for Western blotting, etc.
Silver Staining	I1309	Approx. 1 hour	Several ng	A highly sensitive detection method with a long history of success, capable of detecting DNA.

(When using **C3488**)

1. After electrophoresis, wash gel with 50-100 mL deionized water for 5 minutes a total of three times.
2. Gently mix **C3488** in bottle until homogenous.
3. Remove water after final wash, then immerse gel in **C3488** and shake gently at room temperature for 60 minutes.
4. Remove staining solution and wash with deionized water for at least 60 minutes before observation. If the background is high, wash with deionized water overnight.

(When using **G0615**)

1. After electrophoresis, place gel in a container containing enough deionized water to submerge and shake for 10 minutes.
2. Remove water, add enough Solution A diluted 10-fold with deionized water to submerge gel, and shake gently for 5 minutes.
3. Remove solution and wash gel with enough deionized water to submerge, a total of three times for 10 seconds each.
4. Transfer gel to a new container and add enough Solution B diluted 10-fold with deionized water to submerge. Color will begin to develop. As soon as protein bands appear, decanter off solution and wash three times with deionized water before observation.

*After staining, the gel can be decolorized by gently shaking in a tray containing solution C diluted 10 times with deionized water. After decolorization, remove diluted solution C, wash three times with deionized water. Gel can now be used in downstream experiments.

(When using **I1309**)

1. Prepare Fixing Solution, Staining Solution, Developer Solution, and Stop Solution by diluting supplied solutions 100-fold.
2. After electrophoresis, place gel in a container containing 1x Fixing Solution and shake gently for 10 minutes.
3. Remove Fixing Solution and wash three times with enough deionized water to submerge gel, for 10 minutes each wash.
4. Remove water, add 1x Staining Solution, and shake gently for 5 minutes.
5. Remove Staining Solution, add deionized water, and shake gently for 30 seconds.
6. Remove water, add 1x Developer Solution, and shake gently for 30 seconds.
7. Remove old Developer Solution, add fresh Developer Solution, and shake gently until stained image appears (approximately 5 to 10 minutes).
8. Remove old Developer Solution, add fresh Developer Solution, and shake gently until stained image appears (approximately 5 to 10 minutes).
9. Remove Stop Solution and wash three times with enough deionized water to submerge gel for 5 minutes each wash.