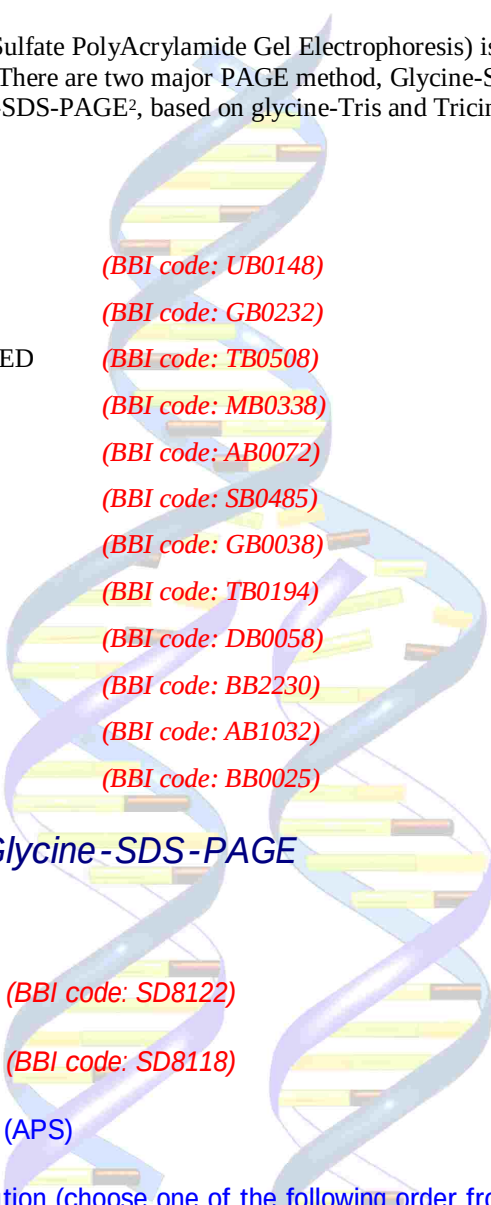


SDS-PAGE Protocol

Introduction

SDS-PAGE (Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis) is commonly used electrophoretic techniques for separating proteins. There are two major PAGE method, Glycine-SDS-PAGE¹ (also know as Laemmli-SDS-PAGE) and Tricine-SDS-PAGE², based on glycine-Tris and Tricine-Tris buffer systems, respectively.

Materials



Urea	(BBI code: UB0148)
Glycerol	(BBI code: GB0232)
Tetramethylethylenediamine, TEMED	(BBI code: TB0508)
Mercaptoethanol	(BBI code: MB0338)
Ammonium persulfate, APS	(BBI code: AB0072)
Sodium Dodecyl Sulfate, SDS	(BBI code: SB0485)
Coomassie blue G-250	(BBI code: GB0038)
Tris base	(BBI code: TB0194)
Dithiothreitol, DTT	(BBI code: DB0058)
Bromophenol blue	(BBI code: BB2230)
Acrylamide	(BBI code: AB1032)
Bis-acrylamide	(BBI code: BB0025)

Buffer Preparation for Glycine-SDS-PAGE

1.5 M Tris-HCl, pH 8.8

0.5 M Tris-HCl, pH 6.8 (BBI code: SD8122)

10% (w/v) SDS (BBI code: SD8118)

10% (w/v) ammonium persulfate (APS)

Acrylamide/Bis-acrylamide Solution (choose one of the following order from BBI)

Acry/Bis Solution (19:1), 30% (w/v) (BBI code: A0009)

Acry/Bis Solution (29:1), 30% (w/v) (BBI code: A0010)

Acry/Bis Solution (37.5:1), 30% (w/v) (BBI code: A0011)



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5x Sample Buffer

Tris-HCl, pH 6.8	0.2 M
SDS	10% (w/v)
Dithiothreitol	10 mM
Glycerol	20% (w/v)
Bromophenolblue	0.05%

(Optional: add urea up to 8M for really hydrophobic proteins)

5x SDS Running Buffer

Tris base	15 g/L
Glycine	72 g/L
SDS	5 g/L

Coomassie Blue Stain

Acetic acid	10% (v/v)
Coomassie Blue Dye	0.006 (w/v)
ddH ₂ O	90%

Guideline for Gel Strength

% Gel	M.W. Range
6-8	50 kDa - 500 kDa
10	20 kDa - 300 kDa
12	10 kDa - 200 kDa
15	3 kDa - 100 kDa

Separating gel Preparation for Glycine-SDS-PAGE

Componets	Volume of componets for different volumes of gels (ml)							
	5	10	15	20	25	30	40	50
6% Gel								
ddH ₂ O	2.6	5.3	7.9	10.6	13.2	15.9	21.2	26.5
30% Acryl/Bis Solution	1.0	2.0	3.0	4.0	5.0	6.0	8.0	10.0
1.5 M Tris pH 8.8	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
10% SDS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.004	0.008	0.012	0.016	0.02	0.024	0.032	0.04
8% Gel								
ddH ₂ O	2.3	4.6	6.9	9.3	11.5	13.9	18.5	23.2
30% Acryl/Bis Solution	1.3	2.7	4.0	5.3	6.7	8.0	10.7	13.3
1.5 M Tris pH 8.8	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5



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10% SDS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.003	0.006	0.009	0.012	0.015	0.018	0.024	0.03
10% Gel								
ddH ₂ O	1.9	4.0	5.9	7.9	9.9	11.9	15.9	19.8
30% Acryl/Bis Solution	1.7	3.3	5.0	6.7	8.3	10.0	13.3	16.7
1.5 M Tris pH 8.8	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
10% SDS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
12% Gel								
ddH ₂ O	1.6	3.3	4.9	6.6	8.2	9.9	13.2	16.5
30% Acryl/Bis Solution	2.0	4.0	6.0	8.0	10.0	12.0	16.0	20.0
1.5 M Tris pH 8.8	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
10% SDS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02
15% Gel								
ddH ₂ O	1.1	2.3	3.4	4.6	5.7	6.9	9.2	11.5
30% Acryl/Bis Solution	2.5	5.0	7.5	10.0	12.5	15.0	20.0	25.0
1.5 M Tris pH 8.8	1.3	2.5	3.8	5.0	6.3	7.5	10.0	12.5
10% SDS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
10% APS	0.05	0.1	0.15	0.2	0.25	0.3	0.4	0.5
TEMED	0.002	0.004	0.006	0.008	0.01	0.012	0.016	0.02

Stacking gel Preparation for Glycine-SDS-PAGE

Componets	Volume of componets for different volumes of gels (ml)							
	1	2	3	4	5	6	8	10
5% Gel								
ddH ₂ O	0.68	1.4	2.1	2.7	3.4	4.1	5.5	6.8
30% Acryl/Bis Solution	0.17	0.33	0.5	0.67	0.83	1.0	1.3	1.7
1.0 M Tris pH 6.8	0.13	0.25	0.38	0.5	0.63	0.75	1.0	1.25
10% SDS	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
10% APS	0.01	0.02	0.03	0.04	0.05	0.06	0.08	0.1
TEMED	0.001	0.002	0.003	0.004	0.005	0.006	0.008	0.01

Buffer Preparation for Tricine-SDS-PAGE

Gel Buffer

Tris-HCl, pH 8.45

3 M

SDS

0.3% (w/v)

Acrylamide/Bis-acrylamide Solution (choose one of the following order from BBI)

Acry/Bis Solution (19:1), 40% (w/v)

(BBI code: A0006)

Acry/Bis Solution (29:1), 40% (w/v)

(BBI code: A0007)



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Acry/Bis Solution (37.5:1), 40% (w/v) *(BBI code: A0008)*

70% Glycerol

10% Ammonium Persulfate, APS

10x Cathode Buffer

Tris	1M
Tricine	1M
SDS	1%

Adjust pH to 8.25

10x Anode Buffer

Tris	2.1M	Adjust pH to 8.9
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5x Sample Buffer

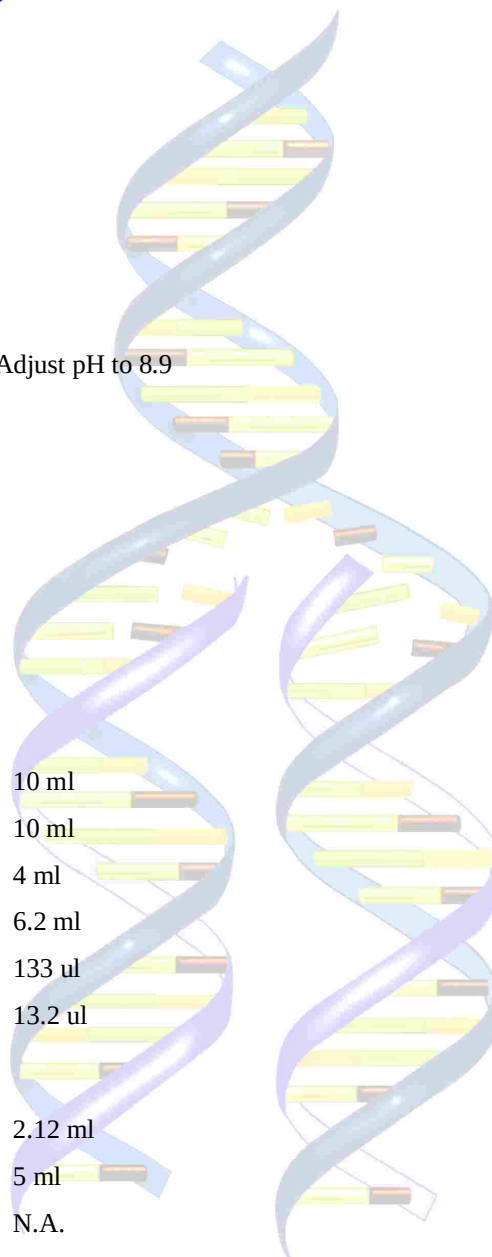
Glycerol	5 ml
SDS	1 g
Mercaptoethanol	2.56 ml
0.5 M Tris-HCl, pH6.8	2.13 ml
Bromophenol Blue	traces

Separating gel

40% Acrylamide Solution	10 ml
Gel Bufer	10 ml
70% Glycerol	4 ml
H2O	6.2 ml
10% APC	133 ul
TEMED	13.2 ul

Stacking gel

40% Acrylamide Solution	2.12 ml
Gel Bufer	5 ml
70% Glycerol	N.A.
H2O	13.44 ml
10% APC	160 ul
TEMED	16 ul





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Reference

1. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685 (1970).
2. Schagger, H. Tricine-SDS-PAGE. *Nat. Protocols* **1**, 16–22 (2006).

