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Product No. PAGE-D
DENATURING, DISCONTINUOUS POLYACRYLAMIDE GEL ELECTROPHORESIS KIT

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INTRODUCTION

Electrophoresis in polyacrylamide gels, in the presence of the anionic detergent sodium dodecyl sulfate (SDS), has proven to be a useful tool for the separation of protein subunits and the determination of their molecular weights.

The molecular weight of a given protein can be determined by comparing its relative mobility with those of known protein markers. An approximately linear relationship is obtained if the logarithms of the molecular weights of standard polypeptide chains are plotted against their relative mobilities (R_f), as described and shown in the Results Section.

PAGE-D KIT

Denaturing, Discontinuous Polyacrylamide Gel Electrophoresis Kit (PAGE-D) contains all of the chemical components needed to prepare a modified Laemmli[1] polyacrylamide gel system. The kit is compatible with Sigma SDS molecular weight markers SDS-6H, SDS-7B, SDS-7, and SDS-6B. Gel porosity may be varied over a wide range to meet specific separation requirements. The components are in convenient containers and only require the addition of deionized water prior to use.

REFERENCES

1. Laemmli, U.K. Nature 227: 680 (1970)

TABLE 1. REAGENTS SUPPLIED IN DENATURING, DISCONTINUOUS POLYACRYLAMIDE GEL ELECTROPHORESIS KIT (PAGE-D)

[RT] Contains enough material for 5 slab gels (16cmx16cm), 15% gel concentration.

Product No.	Material Supplied
B-1773	Buffer, Electrode
B-2148	Buffer, Sample Denaturing 2X Concentrate
B-6148	Buffer, Stacking
B-1898	Buffer, Separating
A-1799	Acrylamide
M-2652	N,N′-Methylene bis-Acrylamide (Bis)
A-1924	Ammonium Persulfate
L-6269	Lauryl Sulfate (SDS)
B-1648	Bromphenol Blue Tracking Dye Solution
M-2527	2-Mercaptoethanol (β-Mercaptoethanol)
L-0269	10% Lauryl Sulfate Solution (SDS)
T-0405	TEMED (N,N,N′,N′-Tetramethylethylenediamine)

Note: Fixing and Staining Components **are not** included in the kit.

DEFINITION OF %T AND %C

$$\text{Total Percent Acrylamide } (\%T) = \frac{\text{grams Acrylamide + grams bis-Acrylamide}}{100 \text{ mL}} \times 100$$

$$\text{Percent Crosslinker } (\%C) = \frac{\text{grams bis-Acrylamide}}{\text{grams Acrylamide + grams bis-Acrylamide}} \times 100$$

PREPARATION OF REAGENTS

All solutions, unless otherwise stated, are stable for at least one month when stored at 0-5°C in tightly closed dark bottles. It is recommended that reagents be warmed to room temperature immediately prior to use.

ELECTRODE BUFFER SOLUTION

Combine the contents of the bottles labeled L-6269 and B-1773 with 2000 mL of deionized water. Stir until completely dissolved. Adjust to a final volume of 2500 mL. This 10X stock solution may be stored in the B-1773 bottle and is stable at room temperature for 4-12 months. (A pressure sensitive label which may be applied to the bottle is supplied for future identification.) Dilute 1 part 10X stock solution with 9 parts deionized water before use. After dilution to 1X, the pH should be in the range of 8.2-8.4. **The kit supplies enough Electrode Buffer Solution for 5 slab gels. The buffer in the lower chamber can be used twice for dual slabs before discarding.**

[Final concentration of 1X Electrode Buffer: 0.025 M Tris-0.192 M glycine and 0.1% lauryl sulfate (SDS), pH 8.3 at 25°C].

STACKING BUFFER SOLUTION

Combine 20 mL of deionized water with the contents of the bottle labeled Buffer, Stacking (B-6148) and stir until dissolved. Adjust to a final volume of 40 mL with deionized water. Buffer may be stored in B-6148 bottle.

[Final Stacking Buffer Concentration: 0.5 M Tris-HCl, pH 6.7-6.9 at 25°C]

SEPARATING BUFFER SOLUTION

Combine the contents of the bottle labeled Buffer, Separating (B-1898) with 20 mL of deionized water and stir until dissolved. Adjust to a final volume of 30 mL with deionized water. (The bottle supplied may be used as a storage container for the Separating Buffer Solution.)

[Final Separating Buffer Concentration: 3.0 M Tris-HCl, pH 8.7-8.9 at 25°C]

ACRYLAMIDE/BIS STOCK SOLUTION

Dissolve the contents of the bottle labeled Acrylamide (A-1799) in 55 mL of deionized water. Then add the contents of the bottle labeled N,N'-Methylene bis-Acrylamide (Bis, M-2652). Rinse the bottle with a small amount of deionized water to remove remaining traces of Bis. Dissolve the Bis completely in the Acrylamide solution and adjust the volume to 100 mL with deionized water. The Acrylamide/Bis stock solution may be stored in the A-1799 bottle. (A pressure sensitive label is supplied for identification.)

[Final Acrylamide:Bis Stock Concentration (29.2:0.8 ratio of Acrylamide:Bis) = 30%T:2.7%C]

AMMONIUM PERSULFATE SOLUTION

To prepare one slab gel, weigh 45 mg of Ammonium Persulfate crystals (A-1924) and add to 3.0 mL of deionized water. **Prepare solution fresh daily.**

[Final Ammonium Persulfate Solution Concentration: 1.5% (w/v)]

2X SAMPLE DENATURING BUFFER SOLUTION

For each milliliter of 2X Sample Denaturing Buffer (B-2148), add 0.1 mL of 2-Mercaptoethanol (M-2527) and 0.002 mL of Bromphenol Blue Tracking Dye Solution. Store frozen (−20°C) in aliquots for long term storage.

[Final 2X sample buffer concentration: 0.125 M Tris-HCl (pH 6.8), 4% Lauryl Sulfate, 10% 2-Mercaptoethanol, 20% Glycerol, 0.004% Bromphenol Blue Dye.]

The following reagents are not supplied with the kit but are needed for post-electrophoresis fixing and staining.

FIXATIVE SOLUTION

Sigma Fixing Solution (F-7264). Dilute according to directions on bottle.
OR
Prepare a solution containing 12% Trichloroacetic Acid and 3.5% 5-Sulfosalicylic Acid dissolved in deionized water.

STAINING REAGENT

Brilliant Blue R Concentrate (B-8647). Dilute according to directions on bottle.
OR
Dissolve 0.5 g Brilliant Blue R (B-0149) in 500 mL of Destaining Solution. Store tightly capped at room temperature. This reagent is stable at room temperature for several months.

Consult the list on pages 10 and 11 for alternate staining reagents available from Sigma.

DESTAINING SOLUTION:

Combine: 400 mL methanol or ethanol
70 mL glacial acetic acid
530 mL deionized water

PREPARATION OF ELECTROPHORESIS GELS

A. Separating Gel

1. Mix Acrylamide/Bis Stock, Separating Buffer, 10% Lauryl Sulfate Stock, Ammonium Persulfate Stock, deionized water (room temperature) and TEMED (T-0405) according to Table 2. AVOID INTRODUCING AIR INTO SOLUTION.

TABLE 2. REAGENT QUANTITIES (mL) FOR GEL PREPARATION

Reagent \ %T	5.0	6.0	7.0	8.0	9.0	10	11	15	20
Acrylamide/Bis Stock	5	6	7	8	9	10	11	15	20
Separating Buffer	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75	3.75
10% Lauryl Sulfate Stock (L-0269)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Deionized Water	19.45	18.45	17.45	16.45	15.45	14.45	13.45	9.45	4.45
----------	Deaerate for 1 minute.----------								
1.5% Ammonium Persulfate Stock	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
TEMED (T-0405)	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
Total Volume	30.015	30.015	30.015	30.015	30.015	30.015	30.015	30.015	30.015

2. Carefully dispense required volume of Solution from Step 1 into gel casting unit.
3. Before the gels polymerize, layer deionized water on top of the Separating Gel. Care must be taken not to disturb the surface of the gel.
4. Allow 30-60 minutes for complete polymerization.

B. Stacking Gel (3.8% T, 2.7%C)

1. To prepare Stacking Gel Solution for 1 slab gel, combine the following:

5.0	mL Stacking Buffer Stock Solution
2.5	mL Acrylamide/Bis Stock Solution
0.2	mL 10% Lauryl Sulfate Stock
11.3	mL deionized water (room temperature) (Deaerate for 30-60 seconds.)
0.015	mL TEMED (T-0405)
1.0	mL 1.5% Ammonium Persulfate Stock
20.015	Total Volume

2. Decant water layer from the polymerized Separating Gel.
3. Wash top of polymerized Separating Gel twice with deionized water.
4. Carefully dispense Stacking Gel Solution onto the top of the polymerized separating gel.

5. Insert comb and bring Stacking Gel Solution to the top of the casting unit.
6. Allow gels to polymerize for 30-60 minutes at room temperature.
7. At this point gels may be used immediately or stored overnight (no longer than 24 hours) at 0-5°C.

PREPARATION OF SAMPLE

Usually a final protein concentration of 0.5 — 1.0 mg/mL in 1X Denaturing Sample Buffer Solution is suitable. The protein solutions should not contain potassium or high concentrations (i.e. greater than 0.2 Molar) of salts. Brief dialysis (2-4 hours) against 0.1% NaCl should render the solution suitable for dilution with the 2X Sample Buffer. Add one part 2X Sample Denaturing Buffer to one part 2X aqueous protein solution to prepare final sample solution.

All proteins must be either incubated at 37°C for 2 hours or boiled for 3-5 minutes in 1X Denaturing Sample Buffer prior to electrophoresis. Aliquots may be frozen for future use. Sample size of 10 μL per well is a typical load level.

[Final 1X Sample Denaturing Buffer concentration:0.0625 M Tris-HCl (pH 6.8), 2% Lauryl Sulfate, 5% 2-Mercaptoethanol, 10% Glycerol and 0.002% Bromphenol Blue Dye.]

ELECTROPHORESIS OF GELS

1. Place the gel into the electrophoresis apparatus.
2. Carefully fill compartments of electrophoresis apparatus with 1X Electrode Buffer Solution. Remove air bubbles trapped under or over gel to prevent distortions in the band(s).
3. Underlay approximately 10 micrograms of protein per well.

   Ten micrograms are recommended for initial trials. It is advised, however, that investigators determine the appropriate amount of protein to be applied for their particular use.

4. Apply constant current (e.g. approx. 30 mA per standard size slab gel, 16 cm x 16 cm x 0.75 mm) until Bromphenol Blue Tracking Dye is 1 centimeter from the anodic end of the gel.

5. Remove gel from slab sandwich and mark the position of the tracking dye

FIXING, STAINING AND DESTAINING

1. Immerse gel in Fixative Solution for 30 minutes.
2. Stain gel in Staining Solution for at least 6 hours with gentle shaking. Overnight staining is preferred.
3. Destain in Destaining Solution. Several changes of Destaining Solution are required.

   Note: Gel should not be destained in the Destaining Solution for longer than 24 hours since some protein bands fade. A 20-24 hour destain time is recommended.
4. Transfer the gel to 7% acetic acid solution for storage. Allow gel to equilibrate in acetic acid solution for at least three hours before reading migration distances. This will allow the gel to fully reswell and prevent further destaining.
5. Record the migration distances of the tracking dye and the blue protein bands from the top of the separating gel.

RESULTS

1 To determine Relative Mobility (R_f) of a protein, divide its migration distance from the top of the separating gel to the middle of the protein band, by the migration distance of the Bromphenol Blue Tracking Dye from the top of the separating gel.

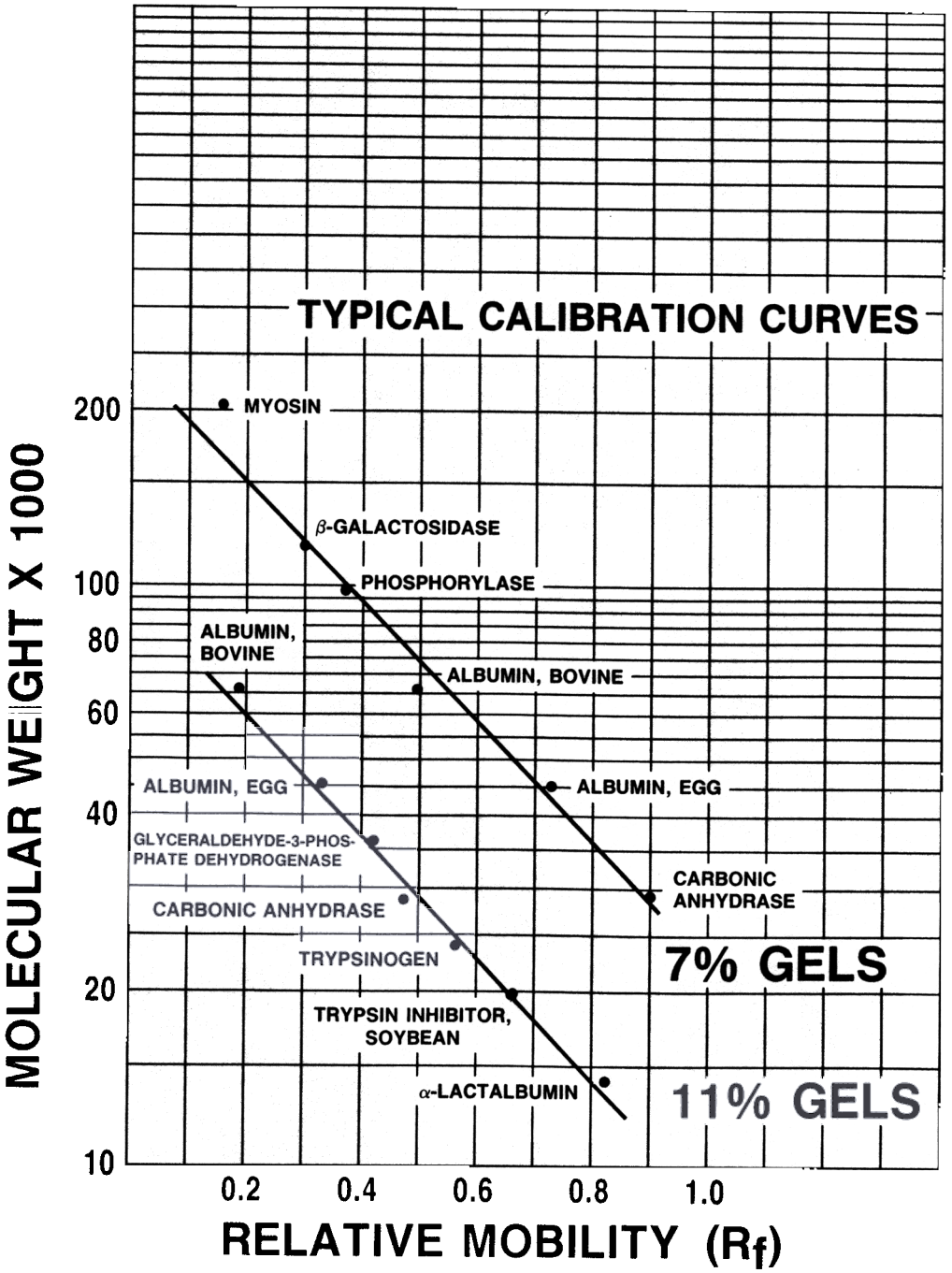
$$R_f = \frac{\text{Distance of Protein migration}}{\text{Distance of Tracking Dye migration}}$$

2 The R_f values (abscissa) are plotted against known molecular weights (ordinate) on semilogarithmic paper to obtain a calibration curve. Each laboratory should prepare its own calibration curve.

3. Estimate the molecular weight of unknown protein from calibration curve

Graph 1

NOTE: The typical calibration curves depicited in the bulletin cannot be used to derive laboratory test results. Each laboratory must prepare its own calibration curve.



TROUBLESHOOTING GUIDE

Problem	Possible Cause	Remedy
Gel doesn't polymerize	1. Too little or no Catalyst (e.g. Ammonium Persulfate or TEMED) 2. Failure to deaerate 3. Temperature too low 4. Ammonium Persulfate not freshly prepared	1. Increase both by two-fold 2. Deaerate 1-2 min. 3. Cast at room temperature, warming glass plates and reagents to room temperature 4. Prepare solution fresh daily
Swirls in Gel	1. Excessive catalysis, gel polymerized in less than 10 minutes 2. Polymerization time greater than 1 hour	1. Reduce Catalyst by one half 2. Increase Catalyst by two-fold. Deaerate.
Gel feels soft	1. Too little cross-linker.	1. Make sure proper %C
Gel turns white	1. Bis concentration too high.	1. Recheck solutions or weights.
Gel brittle	1. Cross-linker too high	1. Make sure proper %C. Usually 4-5%C for 4-6%T gel. 2.5-4%C for 7-12%T gel. 2-2.5%C for 12-20%T gel.
Diffuse or Broad Bands	1. Incomplete catalysis. 2. Sample not equilibrated or high salt. 3. Excessive catalyst. 4. SDS or sample buffer too old.	1. Wait 30-60 min. after pouring stacking gel or increase catalyst. 2. Equilibrate sample to running conditions. 3. Reduce catalyst by one-half. 4. Prepare fresh solutions.
Inconsistent Relative Mobilities	1. Incomplete catalysis or excessive catalyst. 2. Did not deaerate.	1. TEMED and Ammonium Persulfate should be 0.05%. 2. Deaerate 1-2 minutes.