

## Initiators

Chemical polymerization is initiated by Ammonium Persulfate and TEMED. They are reactive by nature and may be prone to oxidation or decomposition. APS can be stored desiccated at room temperature for at least 1 year. Solutions should be made fresh daily, since persulfate in solution decomposes rapidly. TEMED can be stored tightly closed and desiccated either at 4 °C or at room temperature for at least 6 months. After 10 to 12 months, there is a significant reduction in activity and an increase in the amount required for polymerization. If refrigerated, TEMED should be warmed to room temperature before opening to avoid the release of condensation into solution.

## Temperature and Oxygen

Oxygen is an inhibitor of free radical polymerization. Proper degassing of the gel solutions immediately prior to pouring the gel is therefore critical for reproducibility. To insure faster and more complete degassing, bring the gel solution to room temperature first, as cold solutions have a greater capacity for dissolved oxygen. Pouring a gel with a cold solution will have a substantial negative effect on the rate of polymerization and on the quality of the resulting gel as well.

## Contaminants in Buffers

Contaminants in buffer reagents, gel additives, and laboratory water can inhibit polymerization. The most common contaminants of these reagents are metals, non-ion buffers, and breakdown products. Partially inhibited polymerization will result in gels with greater porosity than intended, and the molecules will have greater mobilities.

For a more detailed discussion of gel polymerization, request bulletin 1156 from your Bio-Rad Representative, or in the U.S. call 1-800-4BIORAD.

## Section 5 Risk and Safety Information

### Hazardous components

Acrylamide  
N-N'-Methylene-bis-acrylamide

### Risk and safety phrases

May cause cancer. May cause heritable genetic damage. Toxic in contact with skin and if swallowed. Danger of serious damage to health by prolonged exposure. Avoid exposure - obtain special instructions before use. If you feel unwell, seek medical advice and show label where possible.

Kann Krebs erzeugen. Kann vererbare Schäden verursachen. Giftig bei Berührung mit der Haut und beim Verschlucken. Bei längerer Exposition Gefahr ernster Gesundheitsschäden. Exposition vermeiden - vor Gebrauch besondere Anweisungen einholen. Bei Unwohlsein ärztlichen Rat einholen. Wenn möglich dieses Etikett vorzeigen.

Peut causer le cancer. Peut causer des altérations génétiques héréditaires. Toxique par contact avec la peau et par ingestion. Risque d'effets graves pour la santé en cas d'exposition prolongée. Eviter l'exposition - se procurer des instructions spéciales avant l'utilisation. En cas de malaise consulter un médecin (si possible lui montrer l'étiquette).

Può provocare il cancro. Può provocare alterazioni genetiche ereditarie. Tossico a contatto con la pelle e per ingestione. Pericolo di gravi danni per la salute in caso di esposizione prolungata. Evitare l'esposizione - procurarsi speciali istruzioni prima dell'uso. In caso di malessere consultare il medico (se possibile, mostrargli l'etichetta).

### Safety symbol

h

### CAS number

79-06-1 (Acrylamide)  
110-26-9 (N-N'-Methylene-bis-acrylamide)

**RID/ADR number** 6.1/12c UN 2074

**RTECS number** AS 3325000

**EINECS number** 2011737

**WGK number** 3



## Premixed Preweighed Acrylamide/Bis

**19:1, 30 g**      **Catalog Number 161-0120**

**19:1, 150 g**      **Catalog Number 161-0123**

**29:1, 30 g**      **Catalog Number 161-0121**

**29:1, 150 g**      **Catalog Number 161-0124**

**37.5:1, 30 g**      **Catalog Number 161-0122**

**37.5:1, 150 g**      **Catalog Number 161-0125**

**BIO-RAD**

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## Section 1 Preparation Instructions

For the following %T\* stock solutions, add distilled, deionized water in the given amounts and stir for 30 minutes. Solutions should be mixed directly in the bottle to avoid contact with Acrylamide and Bis.

| %T | For the 30 g bottle: |              | For the 150 g bottle: |              |
|----|----------------------|--------------|-----------------------|--------------|
|    | add H <sub>2</sub> O | final volume | add H <sub>2</sub> O  | final volume |
| 50 | 33 ml                | 60 ml        | 162 ml                | 300 ml       |
| 40 | 48 ml                | 75 ml        | 237 ml                | 375 ml       |
| 30 | 73 ml                | 100 ml       | 362 ml                | 500 ml       |

Filtering is not required but is recommended for the highest quality electrophoresis results.

Recommended shelf life for the stock solution is 30 days at 4 °C.

### \*%T and %C

Polyacrylamide gels are described by reference to two characteristics:

- 1) The **total** monomer concentration (%T):

$$\%T = \frac{\text{g Acrylamide} + \text{g Bis-Acrylamide}}{\text{total volume}} \times 100$$

- 2) The crosslinking monomer concentration (%C):

$$\%C = \frac{\text{g Bis-Acrylamide}}{\text{g Acrylamide} + \text{g Bis-Acrylamide}} \times 100$$

In mixing solutions, a 30% w/v stock solution corresponds to 30% T. The %T will vary depending on the amount of water added. The %C remains constant at all dilutions.

## Section 2 Protocols for Sequencing Gel Preparation

### 2.1 Sequencing Gel Stock Solutions

#### Stock Solutions

- Bio-Rad Preweighed Acrylamide/Bis 19:1.30% Solution:**  
Follow instructions above for mixing and storing.
- 10x TBE Buffer, 0.89 M Tris base, 0.89 M Boric acid, 20 mM EDTA:**  
108 g Tris base  
55 g boric acid  
9.3 g Na<sub>2</sub>EDTA-H<sub>2</sub>O  
Bring up to 1 liter in deionized water. The pH of this solution should be 8.3. Do not add acid or base to alter the pH. Alternatively, use Bio-Rad's Premixed TBE Buffer.
- 25% Ammonium Persulfate (APS):**  
Dissolve 0.25 g in 1 ml distilled water. **Make fresh daily.**
- TEMED:**  
Use neat from the bottle.

### 2.2 Sequencing Gel Preparation

|                         | 4%     | 5%     | 6%     | X%       |
|-------------------------|--------|--------|--------|----------|
| 30% Acrylamide/Bis 19:1 | 20 ml  | 25 ml  | 30 ml  | 5(X%)=ml |
| 10x TBE                 | 15 ml  | 15 ml  | 15 ml  | 15 ml    |
| Urea                    | 63 g   | 63 g   | 63 g   | 63 g     |
| TEMED                   | 150 µl | 150 µl | 150 µl | 150 µl   |
| 25% APS                 | 150 µl | 150 µl | 150 µl | 150 µl   |

Adjust the volume to 150 ml with distilled deionized water. Degas before polymerization.

## Section 3 Protocols for Laemmli Gel Preparation

### 3.1 Laemmli Gel Stock Solutions

- Bio-Rad Preweighed Acrylamide/Bis, 30% solution:** Follow instructions above for mixing and storing.

- 1.5 M Tris-HCl, pH 8.8:**  
54.5 g Tris base  
~150 ml distilled water  
Adjust to pH 8.8 with 1 N HCl. Bring volume up to 300 ml with distilled water and store at 4 °C.
- 0.5 M Tris-HCl, pH 6.8:**  
6.0 g Tris base  
~60 ml distilled water  
Adjust to pH 6.8 with 1 N HCl. Bring volume up to 100 ml with distilled water and store at 4 °C.
- 10% SDS (w/v):**  
Dissolve 10.0 g SDS in distilled water with gentle stirring and bring up to 100 ml. Store at room temperature.
- 10% Ammonium Persulfate (APS):**  
Dissolve 100 mg APS in 1 ml distilled water. **Make fresh daily.**
- TEMED:**  
Use neat from the bottle.

### 3.2 Laemmli Gel Preparation

|                           | Stack  |         | Resolving Gel |                    | X% |
|---------------------------|--------|---------|---------------|--------------------|----|
|                           | 4%     | 7.5%    | 12%           |                    |    |
| 30% Acrylamide/Bis 37.5:1 | 3.3 ml | 25 ml   | 40 ml         | 3.3(X%)<br>=(A)*ml |    |
| 0.5 M Tris-HCl, pH 6.8    | 6.3 ml | --      | --            | --                 |    |
| 1.5 M Tris-HCl, pH 8.8    | --     | 25 ml   | 25 ml         | 25 ml              |    |
| 10% SDS                   | 250 µl | 1.0 ml  | 1.0 ml        | 1.0 ml             |    |
| Distilled deionized water | 15 ml  | 48.5 ml | 33.5 ml       | 73.5-(A)*          |    |
| TEMED                     | 25 µl  | 50 µl   | 50 µl         | 50 µl              |    |
| 10% APS                   | 125 µl | 500 µl  | 500 µl        | 500 µl             |    |
|                           | 25 ml  | 100 ml  | 100 ml        | 100 ml             |    |

Degas before polymerization

\* The letter A designates the volume of 30% Acrylamide/Bis 37.5:1 required to produce the specified percent gel (X%).

## Section 4 General Recommendations

### Acrylamide and Bis

These electrophoresis purity reagents can be stored away from direct sunlight, dry, at room temperature (23-25 °C) for at least 1 year. Heat and light may cause autopolymerization. Monomer stock solutions containing Acrylamide and Bis should be stored at 4 °C for no longer than 1 month. During prolonged storage, hydrolysis of Acrylamide to acrylic acid will occur.

†Laemmli, U. K., *Nature*, 227, 680 (1970).