Separating & Stacking Gel Buffers, 4X

Code	Description	Size
M197-500ML	**Separating Gel Buffer, 4X**	500 mL
M198-500ML	**Stacking Gel Buffer, 4X**	500 mL

General Information

VWR Life Science AMRESCO's Separating and Stacking Gel Buffers are 4X concentrates that simplify standard Laemmli-type acrylamide/bisacrylamide gel preparation. When diluted to 1X, the buffers contain Tris and SDS at the typical pH and concentration required for protein gels.

	Separating Gel Buffer	
Component	**4X**	**1X**
Tris, pH 8.8	1.5 M	0.375 M
SDS	0.4%	0.1%

	Stacking Gel Buffer	
Component	**4X**	**1X**
Tris, pH 6.8	0.5 M	0.125 M
SDS	0.4%	0.1%

Storage/Stability

Store at room temperature (18 – 26°C).

Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

Required Materials Not Provided

Acrylamide/bisacrylamide 37.5:1, 40%
Ammonium persulfate, 10%
TEMED
Deionized water
Vertical gel electrophoresis system
Beaker or flask

Protocol/Procedure

SDS-polyacrylamide separating gel preparation

1. Thoroughly clean the glass plates and combs for electrophoresis before assembling the plates in the gel casting stand according to the manufacturer's instructions.
2. Determine the percent monomer desired in the gel and gently mix together the appropriate volumes of deionized water, acrylamide/bisacrylamide 37.5:1 (40%) solution* and Separating Gel Buffer, 4X in a beaker or flask. The amounts shown in the table below will prepare 10 mL of gel solution. **Note: To prepare gel concentrations not shown in the table, or to determine reagent amounts based on using a 30% acrylamide/bisacrylamide 37.5:1 solution, use the calculation below.*

$$V_A = (X)\ (V_G)/P_A$$

V_A = volume acrylamide/bisacrylamide 37.5:1 solution
X = % monomer in gel
V_G = volume of gel casting solution
P_A = % acrylamide solution (i.e. 30 or 40)

	Separating Gel			
Component	**7.5%**	**10%**	**12.5%**	**15%**
Deionized Water	5.625 mL	5.000 mL	4.375 mL	3.750 mL
Separating Gel Buffer, 4X	2.500 mL	2.500 mL	2.500 mL	2.500 mL
Acryalmide/bisacrylamide 37.5:1, 40%	1.875 mL	2.500 mL	3.125 mL	3.750 mL

3. (Optional) Degas the gel solution for 10 minutes under vacuum aspiration for optimal results.
4. Initiate polymerization of the gel solution by adding100 µL of 10% ammonium persulfate and 10 µL of TEMED for every 10 mL of gel solution. Gently swirl to mix and

immediately pour or pipette the gel solution between the glass plates to a level that leaves sufficient empty space for the stacking gel and combs to be added later.

5. Carefully overlay deionized water on top of the gel solution.
6. Allow the gel to polymerize for 15 – 30 minutes.
7. Discard the overlay and gently blot away excess water with filter paper or a lint-free wipe.
8. Prepare the stacking gel solution by gently mixing together the appropriate volumes of deionized water, acrylamide/bisacrylamide 37.5:1 (40%) solution and Stacking Gel Buffer, 4X in a beaker or flask. The amounts shown in the table below will prepare 10 mL of gel solution.

	Stacking Gel	
Component	**4%**	**5%**
Deionized Water	6.500 mL	6.250 mL
Stacking Gel Buffer, 4X	2.500 mL	2.500 mL
Acryalmide/bisacrylamide 37.5:1, 40%	1.000 mL	1.250 mL

1. Initiate polymerization of the gel solution by adding100 µL of 10% ammonium persulfate and 10 µL of TEMED for every 10 mL of gel solution. Gently swirl to mix and immediately pour or pipette the gel solution between the glass plates up to the top edge.
2. Carefully insert the comb and allow the gel to polymerize for 15 – 30 minutes.
3. Once the gel has polymerized, remove the comb and flush the wells with deionized water or running buffer.
4. Perform electrophoresis per standard procedures.

Directions for Use

For Technical Support

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