GIBCOBRL

LIFE TECHNOLOGIES™

SDS-Polyacrylamide Gel System

Cat. No.: 15574-015

Size: 1 system
Store at 4°C.

Description:
The SDS-Polyacrylamide Gel System is a set of premixed solutions containing all the chemicals necessary (except ammonium persulfate) for the preparation of any size or percentage continuous, gradient, or discontinuous, denaturing (SDS) polyacrylamide gel for the separation of proteins according to molecular weight (1,2). The SDS-Polyacrylamide Gel System contains the following components:

1. 40% (w/v) Acrylamide:Bisacrylamide (37.5:1), 500 ml
2. Resolving Gel Buffer Concentrate [1.5 M Tris-HCl, 0.4% (v/v) TEMED, 0.4% (w/v) SDS, (pH 9.0)], 500 ml
3. Stacking Gel Buffer [0.14 M Tris-HCl, 0.11% (v/v) TEMED, 0.11% (w/v) SDS, (pH 6.8)], 500 ml
4. Two applicator bottles

Consult the table for the quantities of components required to prepare 40 ml of 5% to 15% polyacrylamide resolving gel solution and 10 ml of 3.9% polyacrylamide stacking gel solution. Two applicator bottles have been included for your convenience.

NOTE: AMMONIUM PERSULFATE MUST BE PURCHASED SEPARATELY (CAT. NO. 15523-012).

CAUTION: This product is considered hazardous. Please consult the Material Safety Data Sheet for health and safety information.

Quality Control:
The SDS-Polyacrylamide Gel System is used to prepare a 17 × 15 × 0.15 cm discontinuous polyacrylamide gel (3.9% stacking gel, 12.5% resolving gel). Performance is evaluated by using the prepared gel in the electrophoresis of GIBCO BRL Protein Molecular Weight Standards, High Range.

Protocols:

SDS-Polyacrylamide Gel Formulations

Component, Volume (ml)	Resolving Gel [% (w/v)] 5.0%	7.5%	10.0%	12.5%	15.0%	Stacking Gel [3.9% (w/v)]
40% Acrylamide:bis	5.1	7.7	10.3	12.8	15.4	1.0
Resolving buffer	10.0	10.0	10.0	10.0	10.0	--
Stacking buffer	--	--	--	--	--	9.0
Distilled water	24.5	21.9	19.3	16.8	14.2	--
10% Am. persulfate	0.4	0.4	0.4	0.4	0.4	0.05
Total Volume (ml)	40.0	40.0	40.0	40.0	40.0	10.0

Doc. Rev.: 11/10/00

This product is distibuted for laboratory research use only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Life Technologies TECH-LINE℠ [U.S.A. (800) 828-6686].

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Form No. 18057L

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For a 15 × 17 cm slab gel, approximately 20 ml of resolving gel solution is required when using a 0.8-mm spacer, 40 ml for a 1.5-mm spacer and 80 ml for a 3.0-mm spacer. Approximately 5 ml of stacking gel solution is required when using a 0.8-mm spacer, 8 ml for a 1.5-mm spacer, and 16 ml for a 3.0-mm spacer.

1. Assemble the slab gel glass plates.

2. Prepare the desired volume of resolving gel solution by mixing together the appropriate quantity of 40% Acrylamide:Bisacrylamide, Resolving Gel Buffer Concentrate, distilled water and 10% ammonium persulfate. Use the table as a guide. Add the 10% ammonium persulfate solution last. Mix thoroughly.

   NOTE: For your convenience, use the plastic applicator bottles to prepare gel solutions. The bottles may be reused. Wash carefully after each use.

3. Immediately pour the polymerizing resolving gel solution into the space between the previously assembled slab gel glass plates. Avoid trapping air bubbles by tilting the plates at a 45-degree angle. Pour the solution to within 3.0 cm of the top of the plates.

4. Immediately layer the top of the resolving gel solution with approximately 3 ml of water-saturated n-butanol or water to ensure a good interface with the stacking gel. Allow the gel to polymerize for 30 min.

5. Prepare the desired volume of stacking gel solution by mixing together the appropriate quantity of 40% Acrylamide:Bisacrylamide, Stacking Gel Buffer and 10% ammonium persulfate. Use the table as a guide. Add the 10% ammonium persulfate solution last. Mix thoroughly.

6. Decant and blot with 3MM paper any remaining n-butanol or water from the top of the resolving gel. Pour the stacking gel solution into the gel mold. Insert the comb, and allow the gel to polymerize for 30 min.

7. Carefully remove the comb. Place the gel assembly into the electrophoresis apparatus. Add electrophoresis buffer to the upper and lower chambers of the apparatus. Rinse the wells with electrophoresis buffer, and load the prepared protein samples. Run the gel according to the manufacturer's recommendations.

References:

1. Davis, L. G., Dibner, M. D., Battey, J. F. (1986) *Basic Methods in Molecular Biology*, Elsevier Science Publishing Co., New York.
2. Laemmli, U. K. (1970) *Nature 227*, 680.