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8680

4% / 10% Stacking and Separating Protein Gel V.2

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MATERIALS

STEP MATERIALS

⊗ Acrylamide/bis-acrylamide, 40% solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7802 Sigma**

⊗ Distilled Water **Contributed by users**

⊗ Ammonium persulfate **Contributed by users Catalog #A3678**

⊗ TEMED **Bio-Rad Laboratories Catalog #1610801**

⊗ Isopropanol **Contributed by users**

⊗ Acrylamide/bis-acrylamide, 40% solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7802 Sigma**

⊗ Distilled Water **Contributed by users**

⊗ Ammonium persulfate **Contributed by users Catalog #A3678**

⊗ TEMED **Bio-Rad Laboratories Catalog #1610801**

⊗ Acrylamide/bis-acrylamide, 40% solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7802 Sigma**

⊗ Distilled Water **Contributed by users**

⊗ Ammonium persulfate **Contributed by users Catalog #A3678**

⊗ TEMED **Bio-Rad Laboratories Catalog #1610801**

⊗ Isopropanol **Contributed by users**

⊗ Acrylamide/bis-acrylamide, 40% solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7802 Sigma**

⊗ Distilled Water **Contributed by users**

⊗ Ammonium persulfate **Contributed by users Catalog #A3678**

⊗ TEMED **Bio-Rad Laboratories Catalog #1610801**

1 Into a 15 mL centrifuge tube, add 5 mL 2x Separating Gel Buffer

🧴 5 mL

Protocol

NAME

2x Separating Gel Buffer

CREATED BY

Alan J Cone

PREVIEW

1.1 0.75 M HCl (8.8)

1.2 0.2% SDS

2 Add 2.5 mL 40% Acrylamide/bis-acrylamide (29:1)

2.5 mL

Acrylamide/bis-acrylamide, 40% solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7802 Sigma**

3 Add 2.5 mL DI H₂O

2.5 mL

Distilled Water **Contributed by users**

4 50 uL 10% Ammonium persulfate

50 µL

Ammonium persulfate **Contributed by users Catalog #A3678**

5 Add 5 uL TEMED, then invert tube several times to properly mix all components and initiate the polymerization process.

5 µL

TEMED **Bio-Rad Laboratories Catalog #1610801**

6 Pipette 1 mL at a time of the 10% separating gel mixture between the two pieces of glass until you are about 75% of the way full.

- 7 Add a miniature amount of isopropyl alcohol on top of your separating gel so that it levels off (less than 50 μ L), then wait for the gel to polymerize. You will know it has polymerized as you should have some left in your centrifuge tube. If you invert your centrifuge tube and the solution stays stuck on the bottom, then the gel has successfully polymerized.

00:30:00

Isopropanol **Contributed by users**

- 8 To a new 15 mL centrifuge tube, add 2 mL of 2x stacking gel buffer.

2 mL

Protocol

NAME

2x Stacking Gel Buffer

CREATED BY

Alan J Cone

PREVIEW

8.1 0.25 M HCl (6.8)

8.2 0.2% SDS

- 9 Add 0.4 mL 40% acrylamide/bis-acrylamide (29:1)

Acrylamide/bis-acrylamide, 40% solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7802 Sigma**

0.4 mL

- 10 Add 1.6 mL H₂O

1.6 mL

Distilled Water **Contributed by users**

- 11 Add 20 μ L 10% Ammonium Persulfate

🧪 20 µL

🧪 Ammonium persulfate **Contributed by users Catalog #A3678**

- 12 Add 4 µL TEMED and invert tube to properly mix and initiate polymerization.

🧪 4 µL

🧪 TEMED **Bio-Rad Laboratories Catalog #1610801**

- 13 Use a paper towel to remove any leftover isopropanol, then fill the gel the rest of the way up by pipetting in 1 mL of the 4% stacking gel at a time until the top of the glass is reached, and place the comb in, ensuring that no air bubbles are created.

- 14 Let polymerize, then either use or store wrapped in a wet paper towel and plastic at 4 C.

🕒 00:30:00