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

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DOI: dx.doi.org/10.17504/protocols.io.kqgcvtw

Protocol Citation: Alan Cone 2017. 4% / 10% Stacking and Separating Protein Gel. protocols.io
https://dx.doi.org/10.17504/protocols.io.kqgcvtw

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Protocol status: Working

Created: Nov 08, 2017

Last Modified: Feb 23, 2018
# MATERIALS

## STEP MATERIALS

- **Acrylamide/bis-acrylamide, 40% solution**
  - 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**
  - Sigma Aldrich Catalog #A7802 Sigma

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- **TEMED**
  - Bio-rad Laboratories Catalog #1610801
1 Into a 15 mL centrifuge tube, add 5 mL 2x Separating Gel Buffer

1.1 0.75 M HCl (8.8)

1.2 0.2% SDS

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

2.1 2.5 mL

3 Add 2.5 mL DI H₂O

3.1 2.5 mL
4. 50 µL 10% Ammonium persulfate
   - Contributed by users
   - Catalog # A3678

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

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

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**Protocol**

**NAME**

2x Stacking Gel Buffer

**CREATED BY**

Alan J Cone

8.1 0.25 M HCl (6.8)
9. Add 0.4 mL 40% acrylamide/bis-acrylamide (29:1)
   ✗ Acrylamide/bis-acrylamide, 40% solution 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 µL 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