

# 4% / 10% Stacking and Separating Protein Gel

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## Abstract

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## Protocol

### Step 1.

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

 [AMOUNT](#)

5 ml Additional info:

 [PROTOCOL](#)

. [2x Separating Gel Buffer](#)

CONTACT: [Alan Cone](#)

#### Step 1.1.

0.75 M HCl (8.8)

#### Step 1.2.

0.2% SDS

### Step 2.

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

 [AMOUNT](#)

3 ml Additional info:

 [REAGENTS](#)

Acrylamide/bis-acrylamide, 40% solution [A7802 Sigma](#) by [Sigma Aldrich](#)

### Step 3.

Add 2.5 mL DI H<sub>2</sub>O

 [AMOUNT](#)

3 ml Additional info:



## REAGENTS

✓ Distilled Water by Contributed by users

### Step 4.

50 uL 10% Ammonium persulfate



## AMOUNT

50 µl Additional info:



## REAGENTS

✓ Ammonium persulfate [A3678](#) by Contributed by users

### Step 5.

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



## AMOUNT

5 µl Additional info:



## REAGENTS

TEMED [1610801](#) by [Bio-rad Laboratories](#)

### Step 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.

### Step 7.

Add a miniature amount of isopropyl alcohol on top of your separating gel so that it levels off (less than 50 uL), 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.



## REAGENTS

✓ Isopropanol by Contributed by users



## DURATION

00:30:00

### Step 8.

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



## AMOUNT

2 ml Additional info:



## PROTOCOL

• [2x Stacking Gel Buffer](#)

CONTACT: [Alan Cone](#)

### Step 8.1.

0.25 M HCl (6.8)

### Step 8.2.

0.2% SDS

### Step 9.

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



#### REAGENTS

Acrylamide/bis-acrylamide, 40% solution [A7802 Sigma](#) by [Sigma Aldrich](#)

### Step 10.

Add 1.6 mL H<sub>2</sub>O



#### AMOUNT

2 ml Additional info:



#### REAGENTS

✓ Distilled Water by Contributed by users

### Step 11.

Add 20 µL 10% Ammonium Persulfate



#### AMOUNT

20 µl Additional info:



#### REAGENTS

✓ Ammonium persulfate [A3678](#) by Contributed by users

### Step 12.

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



#### AMOUNT

4 µl Additional info:



#### REAGENTS

TEMED [1610801](#) by [Bio-rad Laboratories](#)

### Step 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.

### Step 14.

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

