



Nov 08, 2017

Version 2

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

DOI

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

Alan Cone¹

¹Wright State University

Ju Lab



Alan Cone

Wright State University

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://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>

License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working



Created: November 08, 2017

Last Modified: February 23, 2018

Protocol Integer ID: 8680

Keywords: separating protein gel, protein

Materials

STEP MATERIALS

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

⊗ Distilled Water

⊗ Ammonium persulfate **Catalog #A3678**

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

⊗ Isopropanol

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

⊗ Distilled Water

⊗ Ammonium persulfate **Catalog #A3678**

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

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

⊗ Distilled Water

⊗ Ammonium persulfate **Catalog #A3678**

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

⊗ Isopropanol

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

⊗ Distilled Water

⊗ Ammonium persulfate **Catalog #A3678**

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



Protocol materials

☒ Distilled Water

☒ Isopropanol

☒ Isopropanol

☒ Distilled Water

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

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

☒ Ammonium persulfate **Catalog #A3678**

☒ Distilled Water

☒ Ammonium persulfate **Catalog #A3678**

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

☒ Ammonium persulfate **Catalog #A3678**

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

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

☒ Distilled Water

☒ Ammonium persulfate **Catalog #A3678**

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

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

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

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

☒ Distilled Water

☒ Ammonium persulfate **Catalog #A3678**

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

☒ Isopropanol

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

☒ Distilled Water

☒ Ammonium persulfate **Catalog #A3678**

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

Troubleshooting



- 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 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

- 4 50 uL 10% Ammonium persulfate

 50 µL

 Ammonium persulfate **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

- 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 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

- 11 Add 20 μ L 10% Ammonium Persulfate

20 μ L

Ammonium persulfate **Catalog #A3678**

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

 4 μ L

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