Sep 15, 2021

SDS-PAGE Using TGX Acrylamide Kit--CHEM 584

Ken Christensen[1]

[1]Brigham Young University

In Development Share

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

Ken Christensen
Brigham Young University

ABSTRACT

Prepare and run your SDS-PAGE gel using the Bio-Rad TGX gel system and the mini-PROTEAN gel box.

DOI

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

PROTOCOL CITATION

Ken Christensen 2021. SDS-PAGE Using TGX Acrylamide Kit--CHEM 584. **protocols.io** https://dx.doi.org/10.17504/protocols.io.byakpscw

CREATED

Sep 15, 2021

LAST MODIFIED

Sep 16, 2021

PROTOCOL INTEGER ID

53292

GUIDELINES

Make APS solutions fresh each day for thebest results.

The glass plates must be clean and free of chips. Clean glass plates with ethanol and lint-free cloths before use.

The height of the stacking gel must be at least 2x the height of the sample in the well.

SAFETY WARNINGS

Acrylamide and bisacrylamide are neurotoxins when in solution. Avoid direct contact with the solutions and clean up all spills. Dispose of acrylamide contaminated pipettes, tubes, etc. in the solid unwanted lab material bucket.

Assemble the gel cassette

1 Assemble the gel cassette on the casting stand. We are pouring a 0.75 mm thick gel and the volumes in this protocol are correct for this thickness of the gel.

Watch this YouTube video to familiarize yourself with how to assemble and pour your gel. TGX Stain-Free™ FastCast™ Acrylamide Gel Casting - YouTube

Prepare the gel solutions and pour the gel

2 Prepare the resolving gel acrylamide solution by combining 2 mL of Resolver A & B solutions in a 15 mL tube.

3 Add 2 µL of TEMED and 20 µL of freshly prepared 10% w/v APS to the combined resolver solution and mix well. Use an appropriate pipette to steadily dispense the solution into the prepared gel cassette. Do not let bubbles form of solution mix with air. Fill the cassette to 0.5-1.0 cm below the bottom of the teeth on the comb. Immediately prepare and pour the stacking gel solutions as directed in the next steps.

> You can quickly prepare a fresh solution of APS by adding approximately 100 mg of APS to a microcentrifuge tube, then adding approximately 1000 µL if ddH_2O.

4 Prepare the stacking gel acrylamide solution by combining 1 mL of the stacker A & B solutions in a 15 mL tube.

5 Add 2 µL of TEMED and 10 µL of the freshly prepared 10% w/v APS to the combined stacker solution and mix well. Pipet solution down the middle of the gel cassette, filling to the top of the short plate. Apply slowly and steadily to prevent mixing with the resolving solution. Align and insert the comb in the cassette carefully to prevent air from being trapped under the comb teeth. Allow the gel to polymerize for 30-45 min before electrophoresis.

Prepare your samples and run your gel

6 Use Laemmli sample buffer (4x) to prepare your samples. Your sample wells should hold up to 33 µL. You do not need to add sample buffer to the protein ladder, you just add the 5 µL aliquot of the protein ladder directly to a lane on your gel.

7 Load your gel into the gel box. Here is a YouTube link to watch the process. Quick Tips: How to Load a Mini-PROTEAN TGX Stain-Free Gel for Electrophoresis - YouTube There are new and old gel boxes, so if you are using an older model, you can ask the TA or the Instructor for assistance.

Fill the inner chamber completely with 1x Running Buffer and the outer chamber to the line for the number of gels you are planning to run.

Run your gel between 100-200 V or until the loading dye has just run off the bottom of the gel.

Citation: Ken Christensen (09/15/2021). SDS-PAGE Using TGX Acrylamide Kit--CHEM 584. https://dx.doi.org/10.17504/protocols.io.byakpscw