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NEXT Gel - CHEM 584

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Adapted from the NEXT Gel instructions included with the solution.

Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

General Information: VWR Life Science AMRESCO's NEXT GEL® products for denaturing gel electrophoresis are proprietary, ready-to-pour solutions comprised of acrylamide, bis-acrylamide, gel buffer, and SDS. The unique chemistry of NEXT GEL® eliminates the need for a stacking gel, thus reducing gel preparation time and extending the separation matrix available for electrophoresis, enabling the resolution of small peptides and high molecular weight proteins in the same gel.

NEXT GEL® solutions polymerize upon addition of ammonium persulfate and TEMED and are fully compatible with all standard electrophoresis equipment, SDS-PAGE staining procedures, and downstream applications including 2D electrophoresis, western blot, transfer, protein sequencing, and MALDI analysis. Each NEXT GEL® acrylamide solution is supplied with NEXT GEL® Running Buffer, 20X, which is essential for optimal gel performance.

Materials

MATERIALS

- TEMED **Bio-rad Laboratories Catalog #**1610801
- APS **Sigma Aldrich Catalog #**A-3678
- NEXT Gel Acrylamide Solution **Amresco**
- NEXT Gel Running Buffer **Amresco Catalog #**M259

Safety warnings

Note: Acrylamide is a potent, cumulative neurotoxin that is absorbed through the skin. Always wear appropriate personal protective equipment, including gloves, when pouring and handling gels.

1 Prepare a fresh solution of 10% w/v ammonium persulfate in water. 1 mL is sufficient for many gels.

2 Add 3 µL of TEMED and 30 µL of freshly made 10% w/v ammonium persulfate (APS) to 5 mL of
NEXT Gel solution in a conical tube. Tighten cap and mix immediately by gently inverting. Pour between prepared glass plates, filling to the top.

3 Insert an appropriate comb and allow gel to polymerize for up to 00:30:00 minutes.
No stacking gel required!

4 Remove comb. Rinse wells with water to remove any residual gel pieces.

5 Assemble mini-gel system. Dilute NEXT GEL® Running Buffer, 20X to 1X by diluting 1:20 in deionized water. Prepare sufficient 1x NEXT Gel Running Buffer from a20X stock solution to fill both the anode and cathode chambers. For the Bio-Rad Mini-Gel Tetra System gel apparatus that we use in the lab, this means that you will need 350 mL of 1x Running Buffer for 1 gel and 700 mL of Running Buffer for 2 gels.

6 Prepare molecular weight markers and samples per standard preparation procedures and
load gel wells. Use the 6x SDS Loading Buffer for preparing samples.

7 Run the gel at 150 volts for up to 01:30:00 or until dye reaches
bottom of the gel.

8 Disassemble the gel apparatus and proceed with the downstream application.

9 Remove and stain gel for proteins using the Coomassie Blue staining solution for up to 01:00:00 or overnight. Transfer to nitrocellulose if performing a western blot.

10 For Coomassie Blue stained gels, destain using the Destain solution for up to 1h or to overnight. The addition of a Kimwipe to Destain can enhance the destaining process. Be careful not to destain too long as the protein bands will lighten.

FAQ's

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Frequently Asked Questions

Problem/Question	Cause	Solution
Why is the gel running too slowly?	Incorrect settings on power supply	Electrophoresis should be run at a constant voltage of 150 volts.
	Use of the incorrect running buffer	Use only NEXT GEL® Running Buffer. Use of other running buffers will increase the run time and reduce band resolution.
	Concentration of salt, lipids or nucleic acids in the protein sample are high	Reduce the concentrations of non-protein contaminants using a protein cleanup method.
	Protein overloading	Reduce protein loaded per lane.
Why are the bands in the gel distorted, smiling, or poorly resolved?	Concentration of salt, lipids or nucleic acids in the protein sample are high, increasing electrical resistance and resulting in gel overheating	Reduce the concentrations of non-protein contaminants using a protein cleanup method.
	Incorrect running buffer used	Use only the NEXT GEL® Running Buffer provided in the kit.
	Protein overloading	Reduce protein loaded per lane.
	Sample proteolysis	Include protease inhibitors during purification to minimize degradation and keep samples on ice.
Why is there smearing at the top of the gel?	Irreversible protein precipitation may occur during heating at 100°C in the loading buffer.	Lower the heating temperature to 60 - 70°C.
	Gel concentration is not optimal	Try a different gel concentration.

Why does the mobility of molecular weight markers appear to be different than for Laemmli gels?	NEXT GEL® is a continuous buffer system rather unlike the discontinuous Laemmli SDS-PAGE. The NEXT GEL® resolving area is longer without a stacking gel. NEXT GEL® electrophoresis generates more heat than Laemmli SDS-PAGE.	Mobility on a 7.5% NEXT GEL® is similar to mobility on a 10% Laemmli gel.
Why are low MW proteins diffuse or not visible?	Proteins below 10 kDa are difficult to fix in a gel.	Add fixing or staining solution immediately after gel run is completed. Do not rinse the gel in water or buffer prior to staining or transfer.
What should be done if the gel is too hot during electrophoresis?	NEXT GEL® typically runs hotter than Laemmli SDS-PAGE. However, if running temp is excessively hot, decrease voltage.	Decrease voltage by 25% or more.
Can TG-SDS or other running buffer be used?	No	Use only the provided NEXT GEL® Running Buffer, 20X. Other commonly used electrophoresis buffers will create artifacts in the gel that impair band resolution.
Can Laemmli loading buffer be used with NEXT GEL®?	Yes	NEXT GEL® Sample Loading Buffer, 4X is recommended, but other loading buffers, including Laemmli loading buffer, may be used.
Can gels be poured and stored for a period of time?	Yes	Gels can be stored cold up to one week in a sealed plastic bag with damp paper towels to keep them hydrated.
Is NEXT GEL® compatible with 2D electrophoresis?	Yes	NEXT GEL® is an excellent replacement for conventional SDS-polyacrylamide gels for the molecular weight separation phase of 2DE.

Is NEXT GEL® Transfer Buffer, 10X the only transfer buffer that may be used?	No	NEXT GEL® Transfer Buffer, 10X (M279), Rapid Transfer Buffer, 10X (N789) and conventional transfer buffer (20 mM Tris pH 8, 150 mM Glycine, 20% Methanol) may be used.