

NEXT GEL® *

A Ready-to-Pour Acrylamide Gel for the Electrophoresis of Proteins

<u>Code</u>	<u>Description</u>	<u>Molecular Weight Separation Range</u>	<u>Size</u>
M254-100ML M254-500ML	NEXT GEL® 5% Solution, 1X Includes : NEXT GEL® Running Buffer, 20X	30 – 500 kDa	100 ml 500 ml
M255-100ML M255-500ML	NEXT GEL® 7.5% Solution, 1X Includes : NEXT GEL® Running Buffer, 20X	20 – 300 kDa	100 ml 500 ml
M256-100ML M256-500ML	NEXT GEL® 10% Solution, 1X Includes : NEXT GEL® Running Buffer, 20X	10 – 200 kDa	100 ml 500 ml
M257-100ML M257-500ML	NEXT GEL® 12.5% Solution, 1X Includes : NEXT GEL® Running Buffer, 20X	3.5 – 100 kDa	100 ml 500 ml
M258-100ML M258-500ML	NEXT GEL® 15% Solution, 1X Includes : NEXT GEL® Running Buffer, 20X	2.5 – 100 kDa	100 ml 500 ml
M261-KIT	NEXT GEL® Trial Kit Includes : Each NEXT GEL® Concentration, 30 ml NEXT GEL® Running Buffer, 20X, 250 ml NEXT GEL® Sample Loading Buffer, 4X, 1 ml		1 Kit
M303-KIT	NEXT GEL® Trial Kit, 5% Solution		1 Kit
M304-KIT	NEXT-GEL® Trial Kit, 7.5% Solution		1 Kit
M305-KIT	NEXT GEL® Trial Kit, 15% Solution		1 Kit
N235-1KIT	NEXT-GEL® Trial Kit, 10% Solution		1 Kit
M283-KIT	NEXT-GEL® Trial Kit, 12.5% Solution		1 Kit

*NEXT GEL® products are patent pending.

General Information:

NEXT GEL® is a novel, ready-to-pour SDS polyacrylamide solution that polymerizes into a unique support matrix for the electrophoretic separation of denatured proteins. The proprietary chemistry of NEXT GEL® eliminates the need for a stacking gel and provides ultra-fine band resolution over a wide molecular weight range. The gradient-like properties of the NEXT GEL® matrix slow the rate of progression of proteins through the electrophoretic field enabling the resolution of small peptides and high molecular weight proteins within the same gel. The gels 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® kit consists of a blended acrylamide solution (acrylamide, bisacrylamide, gel buffer and SDS) and a 20X Running Buffer Solution. The NEXT GEL® Running Buffer supplied with the kit is essential for optimal performance. Kits are available at five different acrylamide concentrations and require only the addition of APS and TEMED prior to pouring the gel.

Storage/Stability:

7.5% through 15% NEXT GEL® kits are stable for 1 year at room temperature. The 5% NEXT GEL® kit is stable for 6 months at room temperature.

Application Disclaimer: For Research Use Only. Not for Therapeutic or Diagnostic Use.

Protocol:
Reagents
NEXT GEL® Kit:

- NEXT GEL® solution, 1X (acrylamide, bisacrylamide, SDS, gel buffer)
- NEXT GEL® Running Buffer, 20X
 - ➔ Do not use other running buffers with the NEXT GEL® system. Buffers not formulated for NEXT GEL® will introduce artifacts that impair band resolution.

Required reagents not included in kit:

- TEMED
- Ammonium Persulfate (APS)
- Sample loading buffer (Included with NEXT GEL® Trial Kit (M261-KIT) only)

Caution: Acrylamide is a potent, cumulative neurotoxin that is absorbed through the skin. Always wear gloves when pouring and handling gels.

20X NEXT GEL® Running Buffer is classified as an irritant due to the high salt concentration. The pH is neutral. Rinse with water if spilled on skin.

1. Prepare gel solution.

- Since stacking gels are not used with NEXT GEL® it is necessary to prepare sufficient NEXT GEL® solution to equal the **total** volume of a traditional resolving gel plus the stacking gel.
- For a 10 cm x 10 cm x 0.75 mm mini-gel, pour 10 ml of NEXT GEL® solution into a conical tube. Add 150-180 µl of APS/TEMED Polymerization Tablet (N310-100TAB) stock solution **OR** 60 µl of freshly made 10% Ammonium Persulfate and 6 µl of TEMED per 10 ml of NEXT GEL® solution.
- Tightly cap the tube and gently invert the solution to mix (DO NOT VORTEX!). Immediately pour the solution between the glass plates. (If the NEXT GEL® solution is at room temperature it is not necessary to degas prior to pouring the gel.) The solution should be poured to the top of the plates since stacking gels are not used with the NEXT GEL® system.

2. Immediately insert comb and allow gel to polymerize completely, about 10 to 30 minutes. The 5% NEXT GEL® (M254) may take up to 1 hour to polymerize.
3. Remove comb and rinse wells with water or running buffer to remove any residual gel pieces. Drain wells completely.
4. Assemble gel system and completely fill both anode and cathode chambers with sufficient 1X NEXT GEL® Running Buffer diluted from the supplied 20X stock solution. Please refer to the operations manual for your specific apparatus for volume recommendations. For optimal resolution use only the supplied NEXT GEL® Running Buffer at the recommended 1X dilution.

➔ Do not use other running buffers with the NEXT GEL® system. Buffers not formulated for NEXT GEL® will introduce artifacts that impair band resolution.

5. Sample Preparation

➔ Electrophoresis on NEXT GEL® is sensitive to the amount of protein loaded on the gel. Protein overloading can lengthen the run time and generate band distortions. To optimize results on mini-gels, load about 0.2 µg-1.0 µg per band per lane for Coomassie® Blue staining. For complex protein mixtures such as cell lysates, load about 1.6 µg - 100 µg of protein per lane. Reduce the amount of protein 10 to 100 fold for silver staining.

High concentrations of salt, lipids and nucleic acids in the loading sample can reduce resolution and generate band distortion. Reduce the concentrations of these as much as possible.

- Dilute 1 part NEXT GEL® 4X Sample Loading Buffer (M260) with 3 parts sample. Final protein concentration should be about 0.16 - 10.0 µg/µl for a heterogenous mixture and about 0.02 - 0.1 µg/µl for purified proteins.
- Boil 3-5 minutes in water bath and cool.
- Load 10-20 µl per well for minigels.

Conventional loading buffers such as AMRESCO 2X Protein Loading Buffer (E270) or Laemmli sample buffer may be used according to standard procedures.

6. Run gel at 150 volts for sixty (60) to ninety (90) minutes or until tracking dye reaches bottom of gel.

➔ When switching to the NEXT GEL® system, monitor initial runs to ensure that voltage remains constant. Protein overloading or high concentrations of salt, lipids or nucleic acids can increase electrical resistance that will overheat gels.

7. Disassemble the apparatus and allow gel to cool briefly before removing from plates. Remove gel and stain with standard methods. NEXT GEL® can be stained with all common SDS-PAGE procedures and is fully compatible with downstream applications including 2D electrophoresis, Western blot transfer, protein sequencing and MALDI analysis.
Notes:

- Gel temperatures will be hotter than the standard SDS-PAGE gels because of the higher voltages used during electrophoresis.
- The color of solutions in the kit may turn yellow after a period of months. The discoloration does not interfere with electrophoresis or compromise performance.
- NEXT GEL® must be used with the supplied Running Buffer. Other commonly used buffers will create artifacts in the gel that impair band resolution.
- Gels can be cast and stored up to a week. Store in a sealed plastic bag with damp paper towels to keep gels hydrated.

Tips for downstream applications

- Western blotting:** Blotting procedures can be applied to NEXT GEL® without modification. Efficiency is similar to standard Laemmli gels.

 - AMRESCO NEXT GEL® Transfer Buffer (M279) is formulated to ensure high efficiency transfer from NEXT GEL® to either nitrocellulose or PVDF membranes. Standard transfer buffers (AMRESCO 10X CAPS Transfer Buffer [K972-500ml] or 20 mM Tris, pH 8.0, 150 mM Glycine, 20% methanol) can be used as well. Pre-equilibration of NEXT GEL® in transfer buffer is not necessary.
 - For 0.75 mm mini-gels, transfer at 2-3 amps for 15 to 20 minutes with either semi-dry or immersion-type apparatus. The use of pre-stained markers such as AMRESCO's BlueStep™ Broad Range Protein Marker (K973—0.5ML) is recommended to verify transfer efficiency.
- 2 Dimensional Electrophoresis (2DE):** NEXT GEL® is an excellent replacement for conventional SDS-PAGE gels for the molecular weight separation phase of 2DE. Prepare sufficient NEXT GEL® solution to include the total volume of both the resolving and stacking gel since a stacking gel is not used with the NEXT GEL® system. After casting, water or water-saturated butanol may be used to overlay NEXT GEL®.

Related Products

<u>Code</u>	<u>Product</u>
Required Reagents not Included in Kit	
N310-100TAB	APS/TEMED Polymerization Tablets
0486-25G	Ammonium Persulfate
0761-25ML	TEMED
Special Purpose NEXT GEL® Kits	
M271-KIT	Native NEXT GEL® Electrophoresis Kit
M272-KIT	Large Protein NEXT GEL® Electrophoresis Kit
M281-KIT	HTS (High Throughput) NEXT GEL® Electrophoresis Kit
NEXT GEL® Loading and Transfer Buffers	
M260	NEXT GEL® Sample Loading Buffer, 4X
E270	Loading Buffer, 2X, Protein
M279	NEXT GEL® Transfer Buffer, 10X
Protein Molecular Weight Markers	
J383-200UL	Precise™ Protein Molecular Weight Marker, 7 bands, 15.0-150.0 kDa
K494-500UL	Wide Range Protein Molecular Weight Markers, 8 bands, 14.0-212.0 kDa range
K973—0.5ML	BlueStep™ Broad Range Protein Marker, Pre-Stained

Stains

K217-1L	Blue BANDit™ Protein Stain
M227-1L-KIT	Silver-BULLit™ Staining Kit

Trouble Shooting

Gel is running too slow

- Electrophoresis with the NEXT GEL® system should be run at constant voltage of **150** volts.
- Use of running buffers other than the supplied 20X Running Buffer will double the run time and reduce band resolution.
- High concentrations of salts, lipids or nucleic acids in samples can extend run times. These components should be reduced as much as possible prior to electrophoresis.
- The gel is overloaded with protein. See '**Poor band resolution**' below for guidelines for protein loads.

Gel is too hot during the run

- Reduce the voltage by 25% or more.

5% NEXT GEL® is tearing during post-run handling

- Let gel cool before removing from plate.
- Place plate in container with water or buffer and slowly remove from plate. Keeping gel moist will help prevent tearing.
- If necessary increase gel thickness to 0.75 mm-1.5 mm.

Staining problems

- NEXT GEL® is compatible with all SDS-PAGE staining procedures. Procedures should be performed with continuous shaking.
- Dark or uneven background with Coomassie® Blue.
 - Stain 0.75 mm mini-gels for 30 minutes or less.
 - Destain in 50% methanol, 10% acetic acid for 20 minutes.
 - Destain an additional hour in 12.5% methanol, 2.5% acetic acid.
- High background with Silver Staining
 - Double the volume of wash solution.

Poor band resolution

- Reduce the amount of protein loaded per lane. The following amounts are guidelines for a standard mini-gel.
 - For Coomassie® Blue staining sample concentrations should be about 0.16 - 10.0 µg/µl for a heterogenous mixture and about 0.02 - 0.1 µg/µl for purified proteins.
 - Reduce the amount loaded 10 to 100 fold for silver staining.
 - Reduce the voltage by 25%.
- Minimize the salt, lipid and nucleic acid concentrations in sample.
- Sample proteolysis can generate diffuse or poorly resolved bands. It can occur during purification or during denaturation in the loading buffer since some

proteases are active in SDS. Include protease inhibitors during purification to minimize degradation. After adding loading buffer, keep samples on ice prior to heating at 70° to 100°C for 3-5 minutes.

- Smearing at the top of the gel may arise from irreversible protein precipitation during heating at 100°C in loading buffer. Lower heating temperature to 60° to 70°C.
- Try a different gel concentration. Recommended NEXT GEL® concentrations for a given molecular weight range are listed below.

NEXT GEL® Concentration	Molecular Weight Separation Range
5%	30-500 kDa
7.5%	20-300 kDa
10%	10-200 kDa
12.5%	3.5-100 kDa
15%	2.5 -100 kDa

Band smiling, smearing or distortion

- Decrease voltage in the first 15 minutes by 25%.
- Reduce amount of salt, nucleic acids, or lipids in the sample.
- Reduce the amount of total protein. (See guidelines above in the section concerning poor band resolution).
- Run gel at 100 volts for 15 minutes. Increase to 150 volts for 1-1.5 hours until tracking dye reaches bottom.

Mobility of markers is different from Laemmli gels

- The mobility of individual bands in molecular weight markers may vary relative to Laemmli gels. The band order will be the same. These changes arise from several differences in the NEXT GEL® system relative to Laemmli gels.
 - NEXT GEL® is based on a continuous buffer system rather than the discontinuous Laemmli SDS-PAGE.
 - The gel is a couple centimeters longer because of the absence of the stacking gel.
 - NEXT GEL® runs generate more heat than Laemmli SDS-PAGE.
 - Mobility on a 7.5% NEXT GEL™ is similar to a 10% Laemmli gel.

Low MW proteins not visible or diffuse

- 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.

References:

1. Andrews, A.T. Electrophoresis: Theory, Techniques, and Biochemical and Clinical Applications 2nd ed., New York , (1988), 21-24.
2. Ogden, R.C. and Adams, D.A. Electrophoresis in agarose and acrylamide gel. Methods Enzymol., 152, 61-87 (1987)

3. Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680-685.

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