VWR AMRESCO LIFE SCIENCE

Directions for Use

NEXT GEL®

Code	Description	Size
M255-100ML	**NEXT GEL® 7.5%**	100 mL
M255-500ML	**NEXT GEL® 7.5%**	500 mL
M256-100ML	**NEXT GEL® 10%**	100 mL
M256-500ML	**NEXT GEL® 10%**	500 mL
M257-100ML	**NEXT GEL® 12.5%**	100 mL
M257-500ML	**NEXT GEL® 12.5%**	500 mL
M258-100ML	**NEXT GEL® 15%**	100 mL
M258-500ML	**NEXT GEL® 15%**	500 mL

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 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.

- Ready-to-pour SDS polyacrylamide solutions
- Faster gel casting with no stacking gel required
- Broad range of separation – 3.5 kDa and 212 kDA on the same gel
- Stable > 1 year at room temperature
- NEXT GEL® Running Buffer, 20X included with purchase

Storage/Stability

Store at room temperature (18 - 26°C). Stable for at least one year.

Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

Supplied Materials

Kit Code	Component Code	Description
M255	1B1723	NEXT GEL® 7.5%,
	M259	NEXT GEL® Running Buffer, 20X
M256	1B1724	NEXT GEL® 10%
	M259	NEXT GEL® Running Buffer, 20X
M257	1B1726	NEXT GEL® 12.5%
	M259	NEXT GEL® Running Buffer, 20X
M258	1B1726	NEXT GEL® 15%
	M259	NEXT GEL® Running Buffer, 20X

Required Materials Not Supplied

Ammonium Persulfate (APS)
TEMED
NEXT GEL® Sample Loading Buffer, 4X (M260-5.0ML)

Protocol/Procedure

Gel polymerization and assembly

*__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.

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

1. Prepare a fresh solution of 10% ammonium persulfate in water.
2. For each 10 cm x 10 cm x 0.75 mm mini-gel, pour 10 mL of NEXT GEL® acrylamide solution into a conical tube.
3. Add 60 µL of 10% ammonium persulfate and 6 µL of TEMED per 10 mL of NEXT GEL® solution.
4. Tightly cap the tube and gently invert to mix.
5. Immediately pour the solution to fill the entire volume of the gel casting plates.
6. Insert a comb immediately and allow gel to polymerize completely, about 15 – 30 minutes.
7. Dilute NEXT GEL® Running Buffer, 20X to 1X by diluting 1:20 in deionized water.
8. Assemble the gel system and fill the anode and cathode chambers with sufficient volumes of 1X NEXT GEL® Running Buffer. (Refer to the operations manual for the electrophoresis apparatus for volume recommendations.)
9. Remove the comb from the gel and rinse the wells with running buffer.

Sample preparation and gel electrophoresis

***Note:** For optimal resolution using NEXT GEL® (mini-gel), refer to the guidelines below. Reduce the amount of protein to be loaded 10 to 100-fold if the gel will be used for silver staining.

Sample	Concentration per well	Total amount per well
Purified protein	0.02 – 0.1 µg/µL	0.2– 1.0 µg
Lysate	0.16 – 10 µg/µL	1.6 – 100 µg

1. Dilute 1 part NEXT GEL® Sample Loading Buffer, 4X with 3 parts protein sample.
2. Boil 3 – 5 minutes in a water bath and cool.

3. Load 10 µL per well for mini-gels.
4. Run gel at 150 volts for 60 – 90 minutes or until the tracking dye reaches the bottom of the gel.
5. Disassemble the gel apparatus and proceed with downstream application. If performing a Western blot, 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 for the transfer step.

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.

For Technical Support
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NEXT GEL®
ZY0593
Rev. 1 11/2015