AMRESCO®

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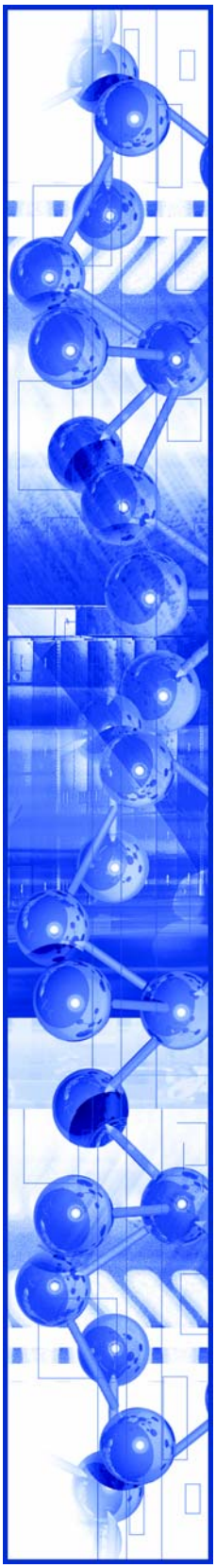
Product Information



FLUORESCENT NEXT GEL™

A Ready-to-Pour Acrylamide Gel Solution Containing a Fluorescent Stain for Rapid Protein Band Visualization after Electrophoresis

Code	Description	MW Separation Range	Size
M290-100ML-KIT M290-500ML-KIT	Fluorescent NEXT GEL™ 10% Solution, 1X Includes : NEXT GEL™ Running Buffer, 20X	10 – 200 kDa	100 ml 500 ml
M291-100ML-KIT M291-500ML-KIT	Fluorescent NEXT GEL™ 12.5% Solution, 1X Includes : NEXT GEL™ Running Buffer, 20X	3.5 – 100 kDa	100 ml 500 ml

**Fluorescent NEXT GEL™ products are patent pending.*

General Information:

Fluorescent NEXT GEL™ is a revolutionary product for SDS-PAGE that provides nearly instantaneous band visualization with ultra-fine band resolution. A proprietary fluorescent reagent included in the acrylamide solution binds to SDS-denatured proteins and co-migrates with them through the gel. Following electrophoresis, the protein-probe complex fluoresces after a brief exposure (less than 5 minutes) to UV light. Background fluorescence is virtually absent since the unbound probe does not emit light upon irradiation. Any need for post-run staining and destaining is completely eliminated. Sensitivity is comparable to Coomassie® Blue staining. Fluorescent NEXT GEL™ is ideal for applications where immediate visualization of protein bands is desired. Its use in downstream procedures such as Western blotting, protein sequencing and MALDI analysis is currently under investigation.

AMRESCO's NEXT GEL™ is 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 so that small peptides and high molecular weight proteins can be resolved within the same gel. The gels are fully compatible with all standard electrophoresis equipment,

Each Fluorescent NEXT GEL™ kit consists of a 1X blended acrylamide solution (fluorescent reagent, acrylamide, bisacrylamide, gel buffer and SDS) and a 20X NEXT GEL™ Running Buffer solution. The NEXT GEL™ Running Buffer supplied with the kit is essential for optimal performance. Kits are available at acrylamide concentrations of 10% or 12.5% and require only the addition of APS and TEMED prior to pouring the gel.

Storage/Stability:

Fluorescent NEXT GEL™ kits are stable for at least 6 months at room temperature. Fluorescent NEXT GEL™ is light sensitive and should be stored in the amber bottle provided.

Application Disclaimer

For Research Use Only.
Not for Therapeutic or Diagnostic Use.

Protocol:

Reagents

Fluorescent NEXT GEL™ Kit:

- Fluorescent NEXT GEL™ solution, 1X (acrylamide, bisacrylamide, SDS, gel buffer)
- NEXT GEL™ Running Buffer, 20X

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

Required reagents not included in kit:

- TEMED
- Ammonium Persulfate (APS)
- Sample loading buffer

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

NEXT GEL™ Running Buffer, 20X 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 Fluorescent NEXT GEL™ it is necessary to prepare sufficient Fluorescent NEXT GEL™ solution to equal the **total** volume of a traditional resolving gel plus the stacking gel.
   - For a 10 cm x 10cm x 0.75 mm mini-gel, pour 10 ml of Fluorescent NEXT GEL™ solution into a conical tube. Add 60 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 Fluorescent 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 Fluorescent 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 Fluorescent NEXT GEL™ system.
2. Immediately insert comb and allow gel to polymerize completely, about 10 to 30 minutes.
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 Fluorescent NEXT GEL™ system. Buffers not formulated for Fluorescent NEXT GEL™ will introduce artifacts that impair band resolution.
5. Sample Preparation

   ➥Electrophoresis on Fluorescent 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 complex protein mixtures such as cell lysates, load about 1.6 µg - 20 µg of protein per lane.

   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.
   - Add protein loading buffer to sample according to standard procedures. NEXT GEL™ Sample Loading Buffer, 4X (M260) is specially formulated for NEXT GEL™, but conventional Laemmli sample buffer such as AMRESCO 2X Protein Gel Loading Buffer (E270) may be used as well. Final protein concentration of a heterogeneous sample should be about 0.16 - 2.0 µg/µl. Purified proteins should be loaded at final concentrations between 0.02 - 0.1µg/µl.
   - Boil 3-5 minutes in water bath and cool.
   - Load 10-20 µl per well for mini-gels.
6. Run gel at **175 volts to 200 volts** for thirty (30) to sixty (60) minutes or until tracking dye reaches bottom of gel.

   ➥When switching to the Fluorescent 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 place on a UV transilluminator for 3-5 minutes until bands are visible. Protein bands will fluoresce as bright white bands against a dark background. Gels may be subsequently stained with Coomassie® Blue (0472-25G) or silver stain (M227-1L-KIT) following standard procedures.

➥Notes:

- Gel temperatures will be hotter than 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.
- Fluorescent NEXT GEL™ must be used with the supplied 20X 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.

Related Products

Code	Product
Required Reagents not Included in Kit	
N310-100TAB	APS/TEMED Polymerization Tablets
0486-25G	Ammonium Persulfate
0761-25ML	TEMED
NEXT GEL™ Loading Buffers	
M260	NEXT GEL™ Sample Loading Buffer, 4X
E270-5ML	Loading Buffer, 2X, Protein
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
Special Purpose Fluorescent NEXT GEL™ Kits	
M272-KIT	L P NEXT GEL™ Kit Resolves large proteins up to 3,000 kDa on a horizontal agarose gel. Fluorescent protocol option is provided.

Trouble Shooting

Gel is running too slow

- Electrophoresis with the Fluorescent NEXT GEL™ system should by run at constant voltage of **175-200** 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.

Poor band resolution

- Reduce the amount of protein loaded per lane. The following amounts are guidelines for a standard mini-gel.
  - Sample concentrations should be about 0.16 - 2.0 µg/µl for a heterogeneous mixture and about 0.02 - 0.1 µg/µl for purified proteins.
  - 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 (AMRESCO Protease Inhibitor Cocktail, General Use, M222-1ML) during purification to minimize degradation. After adding loading buffer, keep samples on ice prior to heating at 70º to 100ºC for 3-5 minutes.
- Smearing at the top of the gel may arise from irreversible protein precipitation during heating at 100ºC in loading buffer. Lower heating temperature to 60º to 70ºC.
- Try a different gel concentration. Recommended Fluorescent NEXT GEL™ concentrations for a given molecular weight range are listed below.

NEXT GEL™ Concentration	Molecular Weight Separation Range
10%	10-200 kDa
12.5%	3.5-100 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 Fluorescent NEXT GEL™ system relative to Laemmli gels.
  - Fluorescent 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.
  - Fluorescent NEXT GEL™ runs generate more heat than Laemmli SDS-PAGE.

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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