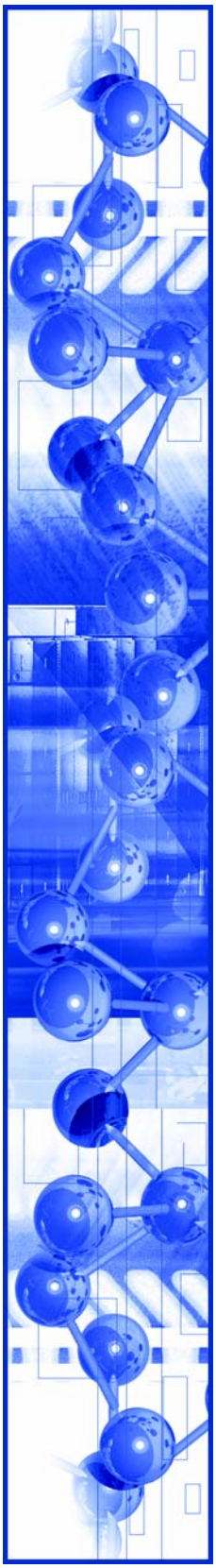

Fluorescent SPRINT NEXT GEL™

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

Code	Description	MW Separation Range (kDA)	Size
M317-KIT-100ML	**Fluorescent SPRINT NEXT GEL™**, **10% Solution**, 1X, 100 ml *Includes :* NEXT GEL™ Running Buffer, 20X, 100 ml x 2 *Sufficient to prepare 10 mini-gels, 10 cm x 10 cm x 0.75 mm*	10 – 200 kDa	100 ml
M317-Kit-500ml	**Fluorescent SPRINT NEXT GEL™**, **10% Solution**, 1X, 500 ml *Includes :* NEXT GEL™ Running Buffer, 20X, 500 ml x 2 *Sufficient to prepare 50 mini-gels, 10 cm x 10 cm x 0.75 mm*	10 – 200 kDa	500 ml
M318-Kit-100ML	**Fluorescent SPRINT NEXT GEL™**, **12.5% Solution**, 1X, 100 ml *Includes :* NEXT GEL™ Running Buffer, 20X, 100 ml x 2 *Sufficient to prepare 10 mini-gels, 10 cm x 10 cm x 0.75 mm*	3.5 – 100 kDa	100 ml
M318-Kit-500ML	**Fluorescent SPRINT NEXT GEL™**, **12.5% Solution**, 1X, 500 ml *Includes :* NEXT GEL™ Running Buffer, 20X, 500 ml x 2 *Sufficient to prepare 50 mini-gels, 10 cm x 10 cm x 0.75 mm*	3.5 – 100 kDa	500 ml

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

General Information:

Fluorescent SPRINT NEXT GEL™ is a novel SDS-PAGE system that enables the entire procedure for standard mini-gels, from gel casting through band visualization, to be performed in under an hour. The supplied SDS polyacrylamide solution requires only the addition of Ammonium Persulfate and TEMED for gel polymerization. It forms a unique support matrix that eliminates the need for a stacking gel while providing ultra fine resolution. A fluorescent dye, incorporated into the acrylamide mixture, binds to the sample proteins as they migrate through the gel. After the run the gel is simply placed on a standard UV transilluminator for 3-5 minutes to visualize bright white bands against a dark background. No post-run staining and destaining is needed. Sensitivity is comparable to Coomassie® Blue and gels can be subsequently stained with Coomassie® Blue if desired. Fluorescent SPRINT NEXT GEL™ is ideal for applications where immediate visualization of protein bands is desired. The gels are fully compatible with all standard electrophoresis equipment, SDS-PAGE staining procedures and downstream applications including and Western blot transfer.

Each Fluorescent SPRINT 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 electrophoresis on the NEXT GEL™ matrix.

Storage/Stability:

FLUORESCENT SPRINT NEXT GEL™ kits are stable for at least 6 months at room temperature. Fluorescent Sprint NEXT Gel ™ is light sensitive and should be stored in the amber bottle provided. All procedures may be performed under ambient light.

Application Disclaimer

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

Protocol:

Reagents

Fluorescent SPRINT NEXT GEL™ Kit:
Fluorescent Sprint NEXT GEL™ solution, 1X
(acrylamide, bisacrylamide, SDS, fluorescent reagent, gel buffer)
NEXT GEL™ Running Buffer, 20X

➥Do not use other running buffers with the Fluorescent SPRINT NEXT GEL™ system. Buffers not formulated for Fluorescent Sprint NEXT GEL™ can result in overheating during the run, impair band resolution and introduce band distortion artifacts.

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. The amounts below are sufficient to prepare a 10 cm x 10 cm x 0.75 mm mini-gel.
   - Since stacking gels are not used with Fluorescent Sprint NEXT GEL™ it is necessary to prepare sufficient Fluorescent Sprint NEXT GEL™ solution to equal the **total** volume of a traditional resolving gel plus the stacking gel.
   - Pour 10 ml of Fluorescent Sprint NEXT GEL™ solution into a conical tube.
   - Add 90 µl of freshly made 10% Ammonium Persulfate and 12 µl of TEMED per 10 ml of Fluorescent Sprint 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 Sprint 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 Sprint NEXT GEL™ system.
2. Immediately insert comb and allow gel to polymerize completely, about 10 to 15 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 Sprint NEXT GEL™ system. Buffers not formulated for the NEXT GEL™ system will introduce artifacts that impair band resolution.
5. Sample Preparation

   ➥Electrophoresis on Fluorescent SPRINT 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. 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.
   - 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 **275 volts to 300 volts** for thirty (30) minutes or until tracking dye reaches bottom of gel.

   ➥When switching to the Fluorescent SPRINT 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 all common SDS-PAGE stains such as Coomassie® Blue (0472-25G) or silver stain (M227-1L-KIT) following standard procedures. Downstream applications including 2D electrophoresis and Western Blotting may be performed 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 SPRINT NEXT GEL™ must be used with the supplied 20X Running Buffer. Other commonly used buffers may generate overheating, impair band resolution and increase band distortion and streaking.
- Gels can be cast and stored up to a week. Store away from light 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 FLUORESCENT SPRINT NEXT GEL™ without modification. Since FLUROESCENT SPRINT NEXT GEL™ can be cast, polymerized and run in less than 45 minutes, an entire Western blot can be performed in a single day.
- AMRESCO NEXT GEL™ Transfer Buffer (M279) is formulated to ensure high efficiency transfer from FLUROESCENT SPRINT 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 SPRINT NEXT GEL™ in transfer buffer is not necessary. Do we need to say anything about including SDS to improve transfer efficiency?
- For 0.75 mm thick mini-gels, transfer at 25V for 30 minutes with a semi-dry transfer 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. **2 Dimensional Electrophoresis (2D):** Fluorescent SPRINT NEXT GEL™ is an excellent replacement for conventional SDS-PAGE gels for the molecular weight separation phase of 2D. Prepare sufficient SPRINT NEXT GEL™ solution to include the total volume of both the resolving and stacking gel since a stacking gel is not used with the SPRINT NEXT GEL™ system. After casting, water or water-saturated butanol may be used to overlay SPRINT NEXT GEL™.

Related Products

Code	Product
Required Reagents not Included in Kit	
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 Sprint NEXT GEL™ system should be run at constant voltage of **275-300** 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 may be 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 - 20.0 µg/µl for a heterogeneous 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 (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 Sprint 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).

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 Sprint NEXT GEL™ system relative to Laemmli gels.
- Fluorescent Sprint 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 SPRINT NEXT GEL™ runs generate more heat than Laemmli SDS-PAGE.

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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