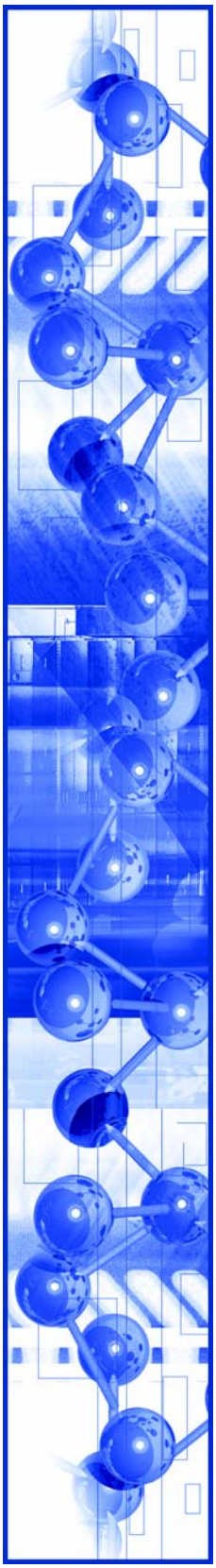

SPRINT NEXT GEL™*

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

Code	Description	Molecular Weight Separation Range	Size
M312-100ML M312-500ML	SPRINT NEXT GEL™, 10% Solution, 1X Includes : NEXT GEL™ Running Buffer, 20X *Each 10 ml will prepare a 10 cm x 10 cm x 0.75 mm mini-gel*	10 – 200 kDa	100 ml 500 ml
M311-100ML M311-500ML	SPRINT NEXT GEL™ 12.5% Solution, 1X Includes : NEXT GEL™ Running Buffer, 20X *Each 10 ml will prepare a 10 cm x 10 cm x 0.75 mm mini-gel*	3.5 – 100 kDa	100 ml 500 ml

**NEXT GEL™ products are patent pending.*

General Information:

SPRINT NEXT GEL™ enables researchers to cast, polymerize and run a mini-gel in less than 45 minutes. Provided as a novel, ready-to-pour SDS polyacrylamide solution, it polymerizes into a unique support matrix for the electrophoretic separation of denatured proteins. The proprietary chemistry of SPRINT NEXT GEL™ eliminates the need for a stacking gel while providing sharp band resolution over a wide molecular weight range. 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 SPRINT 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 two different acrylamide concentrations and require only the addition of APS and TEMED to initiate polymerization prior to pouring the gel.

Storage/Stability:

SPRINT NEXT GEL™ kit is stable for at least 6 months at room temperature. Stability testing is on-going.

Application Disclaimer

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

Protocol:

Reagents

SPRINT NEXT GEL™ Kit:

- SPRINT 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

⚠ **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 SPRINT NEXT GEL™, it is necessary to prepare sufficient SPRINT NEXT GEL™ solution to equal the **total** volume of a traditional resolving gel plus the stacking gel.
   - For a 10 cm x 10 c m x 0.75 mm mini-gel, pour 10 ml of SPRINT NEXT GEL™ solution into a conical tube. Add 90 µl of fresh 10% Ammonium Persulfate solution and 12 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 into the prepared cassette. (If the 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 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 upper and lower 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 SPRINT NEXT GEL™ will impair band resolution as well as increase band distortion and streaking.
5. Sample Preparation

   ➥Electrophoresis on 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 Coomassie® Blue staining. For complex protein mixtures such as cell lysates, load about 1.6 µg - 50 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. Conventional loading buffers such as AMRESCO 2X Protein Loading Buffer (E270) or Laemmli sample buffer may be used according to standard procedures Final protein concentration should be about 0.16 - 5.0 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.
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 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 stain with standard methods. SPRINT 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 may 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.
- SPRINT NEXT GEL™ must be used with the supplied Running Buffer. Other commonly used buffers impair band resolution and increase band distortion and streaking.

- 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

1. **Western blotting**: Blotting procedures can be applied to SPRINT NEXT GEL™ without modification. Since 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 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.
   - For 0.75 mm thick 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. **2 Dimensional Electrophoresis (2D):** 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	
K833-100TAB	Ammonium Persulfate (APS), 100 mg Tablets
0486-25G	Ammonium Persulfate (APS)
0761-25ML	TEMED
NEXT GEL™ Loading Buffers	
M260-5.0ML	NEXT GEL™ Sample Loading Buffer, 4X
E270-5.0ML	Loading Buffer, 2X, Protein
Protein Molecular Weight Markers	
J383-200UL	Precise™ Protein Molecular Weight Marker, 7 bands, 15.0-150.0 kDa range
K972-0.5ML	BlueStep™ Low Range Protein marker, Pre-Stained 6 bands, 19.0 – 120.0 kDa range
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 9 bands, 9.0 – 200.0 kDa range
Stains	
K217-1L	Blue BANDit™ Protein Stain Comassie® based stain with a water destain. *Contains sufficient reagents to stain 50 mini-gels.*
M277-KIT	ZiP™ Reversible Protein Detection Kit Stain the gel, not the bands – Ideal for Western Blotting! *Contains sufficient reagents to stain 25-50 mini-gels.*
M308-125ML	ProLUMA™ Fluorescent Protein Gel Dye Non-toxic, non-hazardous, destain-free fluorescent protein gel stain. Sensitivity comparable to Coomassie® Blue. *Contains sufficient reagents to stain 50 mini-gels.*
Western Blotting Reagents	
M279-500ML	NEXT GEL™ Transfer Buffer, 10X
M282-1L	ProAct™ Membrane Stain Protein membrane stain to confirm successful transfer in Western Blotting.
N218-KIT	VisiGlo™ HRP Chemiluminescent Substrate Kit *Includes: VisiGlo™ HRP Chemiluminescent Substrate A, 120 ml* *VisiGlo™ HRP Chemiluminescent Substrate B, 120 ml* *Sufficient material for ~ 2400 cm^2 of membrane.*
N219-KIT	VisiGlo PLUS™ HRP Chemiluminescent Substrate Kit *Includes: VisiGlo PLUS™ HRP Chemiluminescent Substrate A, 40 ml* *VisiGlo PLUS™ HRP Chemiluminescent Substrate B, 80 ml* *Sufficient material for ~ 2400 cm^2 of membrane.*
N166-1L	ReView™ Stripping & Reprobing Buffer

Trouble Shooting

Gel is running too slow

- Electrophoresis with the SPRINT NEXT GEL™ system should by run at constant voltage of **275-300** volts.
- Use of running buffers other than the supplied 20X Running Buffer will increase the running time, reduce band resolution and increase band distortion and streaking.
- 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.

Staining problems

- SPRINT 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 thick 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.

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 - 5.0 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 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 SPRINT NEXT GEL™ concentrations for a given molecular weight range are listed below.

SPRINT 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 SPRINT NEXT GEL™ system relative to Laemmli gels.
  - 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.
  - 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 protins during the assembly of the head of bacteriophage T4. Nature 227, 680-685.

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