novex®
by life technologies™

NativePAGE™ Sample Prep Kit

Catalog nos.	Product	
BN2005	10% DDM (n-dodecyl-ß-D-maltoside)	**Store at 2ºC to 8ºC**
BN2006	5% Digitonin	**Store at 2ºC to 8ºC**
BN2008	NativePAGE™ Sample Prep Kit	**Store at 2ºC to 8ºC**

Pub. Part no. BN2000.pps MAN0001490 **Rev. Date** 2 December 2011

Description

The NativePAGE™ Sample Prep Kit includes sample preparation reagents for native gel electrophoresis. The kit includes ready-to-use detergent solutions (10% DDM and 5% Digitonin) that improve solubility of hydrophobic and membrane proteins during sample preparation.

The samples prepared with 10% DDM, 5% Digitonin, or the NativePAGE™ Sample Prep Kit are compatible with NativePAGE™ Novex Bis-Tris Gels for native electrophoresis showing increased resolution and reduced streaking.

Kit Contents

BN2005	1 mL 10% DDM (n-dodecyl-ß-D-maltoside)
BN2006	1 mL 5% Digitonin
BN2008	1 mL of 10% DDM; 1 mL of 5% Digitonin; 10 mL of NativePAGE™ 4X Sample Buffer; 0.5 mL NativePAGE™ 5% G-250 Sample Additive

Important

Due to the large diversity of proteins present in different cells and tissues, it is not possible to offer a sample preparation protocol that is suitable for all proteins. Based on the starting material and goal of the experiment, the sample preparation protocol needs to be determined empirically.

Use the brief procedures described on the following pages as a starting point for preparing your sample and then optimize the procedure based on initial results.

Product Use For research use only. Not for human or animal therapeutic or diagnostic use.

Notes

- If a precipitate forms in the 5% Digitonin solution, heat the solution at 95°C for 5 minutes and vortex slowly to dissolve the precipitate. Cool to room temperature prior to use. The 5% Digitonin solution stays in solution at room temperature for up to 1 week.
- You may need to prepare your protein samples with 10% DDM and 5% Digitonin to determine the best solubilizer for your protein.
- If your sample is in a SDS-PAGE sample buffer, prepare a fresh lysate **without** SDS using the detergents included in the sample prep kit. **Do not** use SDS-PAGE samples for native gel electrophoresis.
- Always wear gloves, protective eyewear, and a laboratory coat while handling the detergents.

Materials Needed

- NativePAGE™ 4X Sample Buffer (included with BN2008)
- NativePAGE™ 5% G-250 Sample Additive (included with BN2008)
- Appropriate homogenization unit for tissue samples
- *Optional:* Protease inhibitors and Benzonase nuclease

Prepare Organelle Extracts

Use the following protocol to prepare extracts from isolated organelles, such as chloroplasts or mitochondria.

1. Thaw an aliquot of the isolated, pelleted organelle sample on ice before extraction.
2. Solubilize the organelle proteins in cold 1X NativePAGE™ Sample Buffer containing 0.5–2% DDM or Digitonin. Mix by pipetting up and down and by inversion.
3. Incubate the samples on ice for 15 minutes.
4. Centrifuge the lysates at 20,000 × g for 30 minutes at 4ºC.
5. Aliquot the supernatant into sterile microcentrifuge tubes and store at −80ºC until use. Discard the pellet.

Prepare Cell or Tissue Lysates

1. To 10–50 mg (wet weight) minced tissue, *E. coli* cells, or mammalian cells, add the following to the sample with a final volume of 1 mL:

Reagent	Final Concentration
NativePAGE™ 4X Sample Buffer	1X
10% DDM or 5% Digitonin	1%

2. Homogenize the samples on ice as follows:
   - **For *E. coli***, sonicate the sample **on ice** for 3 rounds of 15 seconds each at ~50% power with cooling the sample on ice between sonications.
   - **For mammalian cells**, pipet the solution up and down several times.
   - **For tissue samples**, use an appropriate homogenization unit.
3. Centrifuge the lysate at 20,000 × g for 30 minutes at 4°C. You may need to use ultracentrifugation for some samples to clarify the lysate.
4. ***Optional:*** Treat high DNA content samples with Benzonase (endonuclease) by adding $MgCl_2$ to a final concentration of 2 mM and 1–2 units Benzonase per µL of sample. Mix well and incubate at room temperature for 30–60 minutes. Centrifuge using the conditions from step 3.
5. Aliquot the supernatant into sterile microcentrifuge tubes and store at –80ºC until use. Discard the pellet.

Determine Protein Concentration

Determine the lysate protein concentration using the Qubit® Protein Assay Kit (Cat. no. Q33211) or BCA protein assay.

Optimize Detergent Concentration

To obtain optimal solubilization of membrane proteins from your samples, optimize the detergent concentrations used based on your initial results.

As a starting point, we recommend using DDM or Digitonin to a final concentration of 1%. For optimal results, you may vary the final DDM concentration from 0.5–5% and final Digitonin concentrations from 0.5–2.5% for your samples. If you need to use higher detergent concentration, you may need to purchase the detergent powder (contact Technical Support for details).

Prepare Samples for NativePAGE™ Gels

After preparing the lysates using detergents and just prior to loading samples onto NativePAGE™ Novex Bis-Tris Gels, add NativePAGE™ 5% G-250 Sample Additive to detergent containing samples on ice such that the final G-250 concentration is 1/4th the detergent concentration. **Do not heat the samples for native gel electrophoresis.**

Troubleshooting Your Sample Preparation Experiments

No bands or a smear observed after electrophoresis

- For protein smearing, decrease the protein load.
- Try varying concentrations of DDM or Digitonin to determine optimal solubilization for your protein sample. You may also need to try other detergents (Schägger, H., 2001 Meth. Cell. Biol. 65: 2311-244).
- If there is a precipitate in the Digitonin solution, heat the solution at 95°C for 5 minutes. Cool the solution to room temperature before use.

Streaking of bands

- Perform benzonase treatment (page 3).
- Remove any particulate material from the lysate using centrifugation or ultracentrifugation.
- Add NativePAGE™ 5% G-250 solution to the detergent containing samples such that the final G-250 concentration is 1/4th the detergent concentration just prior to native gel electrophoresis.

Product Qualification

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at **www.lifetechnologies.com/support**, and is searchable by product lot number, which is printed on each box.

Limited Use Label License: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com

life technologies™