QUICK REFERENCE

Novex® IEF Gels

Pub. Part No. 100031467 **Pub. No.** MAN0003907 **Rev.** A.0

Instructions are provided below for electrophoresis of Novex® IEF Gels using the XCell *SureLock®* Mini-Cell. For details, refer to the *Novex® Technical Guide* available at www.lifetechnologies.com/manuals or contact Technical Support.

Prepare Samples

Reagent	Sample
Sample	*x* µL
IEF Sample Buffer pH 3–10 or pH 3–7 (2X)	5 µL
Deionized Water	to 10 µL final

Prepare 1X Buffer

1X IEF Anode Buffer: Add 20 mL 50X IEF Anode Buffer to 980 mL deionized water. Chill to 4°C to 10°C.

1X IEF Cathode Buffer: Add 20 mL IEF Cathode Buffer pH 3–10 (10X) or pH 3–7 (10X) to 180 mL deionized water. Chill to 4°C to 10°C.

Load Sample

Load the appropriate concentration and volume of your protein on the gel.

Add Buffer

Fill the Upper Buffer Chamber with **chilled** 200 mL 1X IEF Cathode Buffer and the Lower Buffer Chamber with **chilled** 600 mL 1X IEF Anode Buffer.

Run Conditions

Voltage:	100 V constant for 1 hour
	200 V constant for 1 hour
	500 V constant for 30 minutes
Expected Current:	7 mA/gel (start); 5 mA/gel (end)

Stain Gel

Fix the IEF gel in 12% TCA or 12% TCA containing 3.5% sulfosalicylic acid for 30 minutes. Stain the IEF gel with method of choice.

For Research Use Only. Not for use in diagnostic procedures.

Novex® IEF Gels

Prepare for 2D SDS/ PAGE

1. Stain and destain the IEF gel. Incubate the IEF gel in 100 mL 20% ethanol for 10 minutes.
2. Cut out the desired lane (strip) from the gel for transfer to a SDS gel.
3. Incubate the gel strip in 2 mL 2X SDS sample buffer and 0.5 mL ethanol for 3–5 minutes. Aspirate the sample buffer and rinse the gel strip with 1X SDS Running Buffer.
4. Fill the SDS gel cassette with 1X SDS Running Buffer.
5. Trim the IEF gel strip to a length of 5.8–5.9 cm.

6a. Transfer the gel strip into a **1.0 mm** SDS gel by sliding the strip into the 2D-well using a gel loading tip. Avoid trapping air-bubbles between the strip and the SDS gel. Wet a piece of thick filter paper (5.8 cm × 4 cm) in SDS Running Buffer and insert the long edge of the paper into the 2D-well to push the gel strip into contact with the SDS gel.

6b. If transferring the gel strip into a **1.5 mm** SDS gel, wet 2 pieces of thin filter paper (5.8 cm × 4 cm) in 1X SDS Running Buffer. Sandwich the gel strip between the two filter papers along the long edge with the edge of the strip protruding ~0.5 mm beyond the paper. Insert the sandwich into the 2D-well of the SDS gel without trapping air bubbles and push the strip down so it is in contact with the SDS gel.

7. Insert gel into the mini-cell, fill the buffer chambers with 1X SDS Running Buffer, and perform SDS-PAGE.
8. After the dye front has moved into the stacking gel (~10 minutes), disconnect power, remove the paper, and resume electrophoresis.

DISCLAIMER: LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

©2015 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

26 February 2015