

Novex[®] IEF Gels

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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 μ L
	IEF Sample Buffer pH 3–10 or pH 3–7 (2X)	5 μ L
	<u>Deionized Water</u>	<u>to 5 μL</u>
	Total Volume	10 μ L
Prepare 1X Buffer	To prepare 1X IEF Anode Buffer, add 20 mL 50X IEF Anode Buffer to 980 mL deionized water. To prepare 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 1X IEF Anode Buffer and 1X IEF Cathode Buffer to 4°C to 10°C.	
Load Sample	Load the appropriate concentration of your protein sample on the gel.	
Load 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 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 using a method of choice.	

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Prepare for 2D SDS/PAGE

1. After staining and destaining 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 onto the gel cassette and into the 2D-well using a gel loading tip with out trapping any air-bubbles. 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 SDS gel. Push the filter paper down so the gel strip makes contact with the SDS gel.
 - 6b. For 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. Prepare a sandwich of the filter papers and the gel strip along the long edge of the paper so the gel strip is sandwiched between the two filter papers 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.
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