NativePAGE™ Novex Bis-Tris Gels

QUICK REFERENCE CARD

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Instructions are provided below for electrophoresis of NativePAGE™ Novex Bis-Tris Gels using the XCell *SureLock*™ Mini-Cell. For detailed instructions, refer to the NativePAGE™ Gel system manual supplied with the gels or available at www.invitrogen.com.

Prepare Samples

Reagent	Sample with detergent	Detergent-free sample
Sample	x µl	x µl
NativePAGE™ Sample Buffer (4X)	2.5 µl	2.5 µl
NativePAGE™ 5% G-250 Sample Additive	0.25-1 µl*	optional
Deionized Water	to 10 µl	to 10 µl

Do not heat samples for native gel electrophoresis.

*Ensure that the final G-250 concentration is 1/4th the detergent concentration.

Prepare Running Buffer

Add 50 ml 20X NativePAGE™ Running Buffer to 950 ml deionized water to prepare 1X NativePAGE™ Anode Buffer. Add 50 ml 20X NativePAGE™ Running Buffer and 50 ml 20X NativePAGE™ Cathode Additive to 900 ml deionized water to prepare 1X NativePAGE™ Cathode Buffer.

Load Sample

Load samples into sample wells filled with 1X NativePAGE™ Cathode Buffer prior to filling the cathode chamber to better visualize the sample wells. Load appropriate concentration of your protein sample on the gel.

Load Buffer

Fill the Upper Buffer Chamber with ~200 ml 1X NativePAGE™ Cathode Buffer. Fill the Lower Buffer Chamber with ~550 ml 1X NativePAGE™ Anode Buffer.

Run Conditions

Voltage:	150 V constant
Run Time:	90-115 minutes (3-12% gel), 105-120 minutes (4-16% gel)
Expected Current:	12-16 mA/gel (start); 2-4 mA/gel (end)

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Staining Protocol

A quick staining protocol for NativePAGE™ Gels using the Coomassie G-250 from the sample additive is described below. The total staining time is ~2-3 hours and sensitivity is ~60 ng BSA.

For additional staining protocols including a more sensitive Coomassie G-250 staining protocol and a western blotting protocol, refer to the NativePAGE™ Gel system manual supplied with the gels or download the manual from www.invitrogen.com.

Step	Protocol	Time
1	Place the gel in 100 ml fix solution (40% methanol, 10% acetic acid) and microwave on high (950-1100 watts).	45 seconds
2	Shake the gel on an orbital shaker. Discard fix.	15 minutes
3	Place the gel in 100 ml destain solution (8% acetic acid) and microwave on high (950-1100 watts).	45 seconds
4	Shake the gel on an orbital shaker until the desired background is obtained.	

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