Novex[®] Zymogram Gels

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Instructions are provided below for electrophoresis of Novex[®] Zymogram 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.

| Reagent | | Sample |
|---------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample | | xμL |
| Tris-Glycine SDS Sam | ple Buffer (2X) | 5 μL |
| Deionized Water | | <u>to 5 µL</u> |
| Total Volume | | 10 µL |
| Do not heat or reduce samples for Zymogram gels. | | |
| Add 100 mL 10X Tris-Glycine SDS Running Buffer to 900 mL deionized water to prepare 1X Tris-Glycine SDS Running Buffer. | | |
| Load the appropriate concentration of your protein sample on the gel. | | |
| Fill the Upper Buffer Chamber with 200 mL and the Lower Buffer Chamber with 600 mL of 1X Tris-Glycine SDS Running Buffer. | | |
| Voltage: Run Time: Expected Current: | 125 V constant 90 minutes (dependent on 30–40 mA/gel (start); 8–12 | 010, |
| | Sample Tris-Glycine SDS Sam Deionized Water Total Volume Do not heat or reduce Add 100 mL 10X Tris- water to prepare 1X T Load the appropriate Fill the Upper Buffer 0 with 600 mL of 1X Tris Voltage: Run Time: | Sample Tris-Glycine SDS Sample Buffer (2X) Deionized Water Total Volume Do not heat or reduce samples for Zymogram g Add 100 mL 10X Tris-Glycine SDS Running Buffer water to prepare 1X Tris-Glycine SDS Running Buffer Load the appropriate concentration of your protection Fill the Upper Buffer Chamber with 200 mL and a with 600 mL of 1X Tris-Glycine SDS Running Buffer Voltage: 125 V constant Run Time: 90 minutes (dependent or |





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| Develop Gel | Dilute Novex[®] Zymogram Renaturing Buffer (10X) and Novex[®] Zymogram Developing Buffer (10X) 1:9 with deionized water. You nee 100 mL of each buffer per 1–2 mini-gels. | | |
|-------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|--|
| | After electrophoresis, remove the gel and incubate the gel in 1X Zymogram Renaturing Buffer for 30 minutes at room temperature with gentle agitation. | | |
| | Decant the Zymogram Renaturing Buffer and add 1X Zymogram Developing Buffer to the gel. | | |
| | Equilibrate the gel for 30 minutes at room temperature with gentle agitation. | | |
| | 5. Decant the buffer and add fresh 1X Zymogram Developing Buffer to the gel. | | |
| | 6. Incubate the gel at 37°C for at least 4 hours or overnight for maximum sensitivity. The optimal result is determined empirically by varying the sample load or incubation time. | | |
| Stain Gel | Zymogram (Blue Casein) 4–16% gels do not require staining. For non-pre- stained Zymogram gels, stain the gels with Colloidal Blue Staining Kit or the SimplyBlue [™] Safestain. Areas of protease activity appear as clear bands against a dark background. | | |
| Sensitivity Level | 10% Zymogram (Gelatin) Gel: 12% Zymogram (Casein) Gel: 4–16% Zymogram (Blue Casein) Gel: | 10^{6} units of collagenase 7 × 10 ⁴ units of trypsin 1.5 × 10 ⁻³ units of trypsin | |
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