10X Zymogram Developing Buffer

1. Catalog No. KZB050

2. Quantity 500 ml

3. Storage & Stability Store at R.T.

4. Description 10X Zymogram Developing Buffer is used for Running Protein samples for Zymogram PAGE analysis on Polyacrylamide gels.

5. Recommended Loading Add 1/10 volume of 10X Zymogram Developing Buffer to 9/10 volume of D.W..

6. Protocol

1. Mix one part sample with one part of Zymogram Sample Buffer (2X) and let stand 10 minutes at room temperature. DO NOT HEAT.
2. Apply samples (typically 10-25 ul) and run the gel with 1X Zymogram Running Buffer according to the following running conditions:

Voltage	125 V constant
Approx. Current	Start 30-40 mA / 1.0mm gel End 8-12 mA / 1.0mm gel
Approx. Run Time	90 minutes

Turn off the power when the dye is migrated to the end of the gel.

3. After running, dilute the Zymogram Renaturing Buffer (10X) 1:9 with deionized water and incubate the gel in the buffer (100 ml for one or two gels) with gentle agitation for 30 minutes at room temperature.
4. Decant the Zymogram Renaturing Buffer and replace with 1X Zymogram Developing Buffer (100 ml for one or two gels). Equilibrate the gel for 30 minutes at room temperature with gentle agitation then replace with fresh 1X Zymogram Developing Buffer and incubate at 37°C for at least four hours. Incubate overnight for maximum sensitivity. Incubation time can be reduced to as little as one hour for concentrated samples. The optimal result can be determined empirically by varying the sample load or incubation time.
5. Staining: Stain with Coomassie Blue R-250 for 30 minutes. For maximum contrast, use a stain concentration of 0.5% (w/v) instead of the usual concentration of 0.1%. Gels should be destained with Destaining solution, or an appropriate Coomassie R-250 destain. Areas of protease activity will appear as clear bands.

*** Buffer composition**

Zymogram Developing Buffer (10x, 1 L)	
Tris Base	60.246 g
HCl	40.45 ml
NaCl	117 g
CaCl	7.35 g
Brii35	2 g
D.W.	Up to 1 L