Orders: orders@novusbio.com
Support: technical@novusbio.com
Web: www.novusbio.com
Protocols, Publications, Related Products, Reviews and more:
www.novusbio.com/NBP2-30124

NBP2-30124 Protocol

Western Blot Protocol for INSTA-Blot Ovary Tissue OncoPair (NBP2-30124)

Note: The INSTA-Blot(TM) PVDF membrane has been dried and must be rehydrated (Step one) prior to use.

Before wetting, it is suggested that the top of the lanes be marked with an ink pen that will not wash off in methanol.

1. Wet the blots with 100% methanol then thoroughly wash with TBST (25 mM Tris-Cl, pH 8.0; 125 mM NaCl; 0.1% Tween 20) twice to remove residual methanol.

2. Incubate the blot for 1 hr with 5% Carnation nonfat dry milk in TBST to block non-specific antibody binding.

3.Incubate the blots with primary antibody in 1% milk/TBST for 1-2 h at RT or overnight at 4 degrees C.

4. After incubation with the primary antibody, wash the blots five times in TBST then incubate with a secondary antibody conjugated to horseradish peroxidase (HRP-conjugated secondary antibodies) for 1-2 h at RT.

5. After five washes with TBST, develop the blots for 5 min using the PicoTectTM Western Blot Chemiluminescent Substrate.

6. Expose the blots to photographic film for an appropriate time period. We normally use Hyper-film(TM)-ECL films and expose for various periods ranging from 10 s to 20 min to visualize the chemiluminescence signal corresponding to the specific antibody-antigen reaction.