INSTA-Blot™ Human Tissues

Catalog No.: NBP2-31378
Contents: One INSTA-Blot™
Description: Ready-to-use PVDF membrane with 11 human tissue lysates (60 μg total protein per lane).
Shipping: Inert gas packaged, sealed in a light proof bag, and shipped at room temperature (RT).
Storage: Store unopened at RT. For long-term, store at –80°C, stable for one year.

Introduction:
INSTA-Blot™ is a ready-to-use PVDF membrane containing denatured protein from cells or tissue lysates. The INSTA-Blot™ Human Tissues NBP2-31378 is a simple and fast solution for screening proteins from various human tissues. We’ve streamlined the western blotting procedure by eliminating the sample acquisition, preparation, SDS-PAGE and electro-blotting steps. With INSTA-Blot™, immunoblotting is an easy six-step procedure: blocking, primary antibody incubation, wash, secondary antibody incubation, wash, and develop.

Preparation:
INSTA-Blot™ Human Tissues NBP2-31378 contains denatured proteins from human lysates loaded at 50 μg (total protein, Bradford Assay) per lane on a 4-20% Tris-Glycine mini gel. After resolution by SDS-PAGE at 125V for 1 hour the proteins are electro-blotted for 2 hours at 25V onto a PVDF membrane. The membrane is stained with amido black for visualization of proteins, dried and packaged under anoxic conditions. The INSTA-Blot™ is sold ready-to-use in your western blot protocol.

Tissue Collection and Lysis:
Tissues are from donors with no known disease. Tissues are lysed in lysis buffer (10 mM Tris, pH 8.0, 130 mM NaCl, 1% Triton X-100, 10 mM NaF, 10 mM NaPi, 10 mM NaPPI) containing Protease Inhibitor Cocktail (PIC) and PMSF. Prior to SDS-PAGE the tissue lysate is resuspended 1:1 with 2X SDS sample buffer (120 mM Tris-HCl [pH 6.8], 20 mM EDTA, 4% SDS, 0.06% Bromophenol Blue, 20% glycerol, 0.4% β-mercaptoethanol).

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

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 h 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°C.

Research purposes only. Not for diagnostic use.
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; 1:1000-10000 dilution; Novus*) for 1-2 h at RT.

5. After five washes with TBST, develop the blots for 5 min using the PicoTect™ Western Blot Chemiluminescent Substrate (Novus NBP2-29912).

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

**Product Citations:**


**Related Products:**

*HRP Secondary Abs

  - Goat Anti-Mouse Ig HRP Conjugate (# NBP2-30347)
  - Goat Anti-Rat Ig HRP Conjugate (# NBP2-30338)
  - Goat Anti-Rabbit Ig HRP Conjugate (# NBP2-30348)
  - Donkey Anti-Goat IgG (H+L) HRP Conjugate (# NBP2-27510)

**PicoTect™ Western Blot Chemiluminescent Substrate**

(Cat. No. NBP2-29912)

**INSTA-Blot™**

- Rat Tissues (Cat. No. NBP2-30112)
- Human Tissues (Cat. No. NBP2-30113)
- Human Cell Lines (Cat. No. NBP2-30114)
- Multi-species Brain, Testis and Ovary Tissues (Cat. No. NBP2-30115)
- Multi-species Skeletal Muscle, Heart, Kidney Tissues (Cat. No. NBP2-30116)
- Multi-species Liver, Lung, Spleen Tissues (Cat. No. NBP2-30117)
- Multi-species Stomach, Small Intestine, Pancreas Tissues (Cat. No. NBP2-30118)

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