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INSTA-Blot™ Human Tissues

Catalog No.: IMB-103

Contents: One INSTA-Blot™

Description: Ready-to-use PVDF membrane with 11 human tissue lysates (20 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:

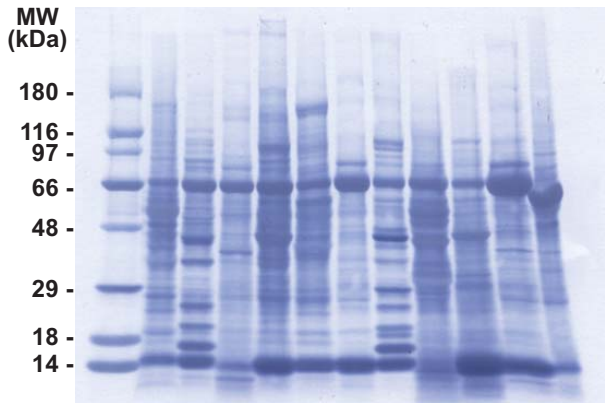
INSTA-Blot™ is a ready-to-use PVDF membrane containing denatured protein from cells or tissue lysates. The INSTA-Blot™ Human Tissues IMB-103 is a simple and fast solution for screening proteins from various human tissues. We've streamlined the western blotting procedure by eliminating the sample aquisition, 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 IMB-103 contains denatured proteins from human lysates loaded at 20 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™ Human Tissues IMB-103. Approximately 20 µg per lane of human tissue lysates are resolved by SDS-PAGE, transfered onto PVDF membrane and stained with amido black. Lanes: 1. Molecular weight marker; 2. Brain; 3. Heart; 4. Small Intestine; 5. Kidney; 6. Liver; 7. Lung; 8. Skeletal Muscle; 9. Stomach; 10. Spleen; 11. Ovary; 12. Testis.

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.

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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; IMGENEX*) 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 (IMGENEX 10087K).

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:

1. Expression, Tissue Distribution, and Cellular Localization of the Antiapoptotic TIP-B1 Protein. Erica S. Berleth, Patricia A. Masso-Welch, Latif A. Kazim, Margot M. Ip, Enrico Mihich, and M. Jane Ehrke. *J. Leukoc. Biol*., 69: 995-1005 (2001).

2. Expression of Placental Leucine Aminopeptidase/Oxytocinase in Neuronal Cells and its Action on Neuronal Peptides. Hideko Matsumoto, Tetsuro Nagasaka, Akira Hattori, Tomohiro Rogi, Nobuo Tsuruoka, Shigehiko Mizutani, and Masafumi Tsujimoto. *Eur. J. Biochem*., 268: 3259-3266 (2001).

3. XPLN, a Guanine Nucleotide Exchange Factor for RhoA and RhoB, But Not RhoC. William T. Arthur, Shawn M. Ellerbroek, Channing J. Der, Keith Burridge, and Krister Wennerberg. *J. Biol. Chem*, 277: 42964-42972 (2002).

4. Alternative Splicing Variants of the Human DBL (MCF-2) Proto-Oncogene. Koichiro Komai, Rie Okayama, Michinori Kitagawa, Hirofumi Yagi, Kazuo Chihara and Shunichi Shiozawa. *Biochemical and Biophysical Research Communications*, 299 (3): 455-458 (2002).

5. Bifunctional Apoptosis Inhibitor (BAR) Protects Neurons from Diverse Cell Death Pathways. W Roth, P Kermer, MKrajewska, K Welsh, S Davis, S Krajewski, J C Reed. *Cell Death and Differentiation* 10, 1178 - 1187 (2003).

6. Cha, a Basic Helix Loop Helix Transcription Factor involved in the regulation of Upstream Stimulatory Factor Activity. Clara I. Rodríguez, Núria Gironès and Manuel Fresno. *J. Biol. Chem*., Vol. 278 (44): 43135-43145, (2003).

7. SGEF, a RhoG Guanine Nucleotide Exchange Factor that Stimulates Macropinocytosis. Shawn M. Ellerbroek, Krister Wennerberg, William T. Arthur, Jill M. Dunty, Dan R. Bowman, Kris A. DeMali, Channing Der, and Keith Burridge. *Mol. Biol. Cell.* (2004) 10.1091/mbc.E04-02-0146.

8. Genomic Organization, Promoter Activity and Expression of the Human Choline Transporter Like Protein 1. Zongfei Yuan, Angela Tie, Mark Tarnopolsky, and Marica Bakovic. *Physiological Genomics*. (2006) 10.1152/physiolgenomics.00107 (2005) (Figure 8a & b).

9. Distribution of CESP-1 Protein in the Corneal Endothelium and Other Tissues. Rieko Kinouchi, Tadatoshi Kinouchi, Toshirou Hamamoto, Takakazu Saito, Adriano Tavares, Tadahiko Tsuru, and Satoru Yamagami. *Invest. Ophthalmol. Vis. Sci*. 47: 1397-1403 (2006).

10. Choline Transport for Phospholipid Synthesis. Michel V, Yuan Z, Ramsubir S, Bakovic M. *Exp Bio Med*. 231:490-504 (2006) (Figure 4).

11. Membrane localization and pH-dependent transport of a newly cloned organic cation transporter (PMAT) in kidney cells. L Xia, K Engel M Zhou, and J Wang. Am J Physiol Renal Physiol 292:682-690 (2007). Imgenex Products cited: 1. INSTA-Blot™ Human Tissue: ready-to-use WB (IMB-103); 2. GAPDH (IMG-5019A-1/IMG-5019A-2). [WB: Fig 2, human kidney tissue]

Related Products:

***HRP Secondary Abs**

Goat Anti-Mouse Ig HRP Conjugate (Cat. No. 20101)

Goat Anti-Rat Ig HRP Conjugate (Cat. No. 20201)

Goat Anti-Rabbit Ig HRP Conjugate (Cat. No. 20301)

Donkey Anti-Goat IgG (H+L) HRP Conjugate (Cat. No. 20401)

PicoTect™ Western Blot Chemiluminescent Substrate
(Cat. No. 10087K)

INSTA-Blot™

Mouse Tissues (Cat. No. IMB-101)

Rat Tissues (Cat. No. IMB-102)

Human Cell Lines (Cat. No. IMB-105)

Multi-species Brain, Testis and Ovary Tissues (Cat. No. IMB-120)

Multi-species Skeletal Muscle, Heart, Kidney Tissues (Cat. No. IMB-121)

Multi-species Liver, Lung, Spleen Tissues (Cat. No. IMB-122)

Multi-species Stomach, Small Intestine, Pancreas Tissues (Cat. No. IMB-123)

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