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INSTA-Blot™ Rat Tissues

Catalog No.: IMB-102

Contents: One INSTA-Blot™

Description: Ready-to-use PVDF membrane with 11 rat 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:

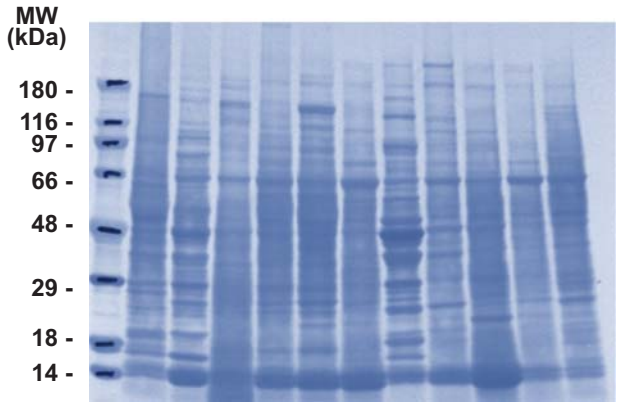
INSTA-Blot™ is a ready-to-use PVDF membrane containing denatured protein from cells or tissue lysates. The INSTA-Blot™ Rat Tissues IMB-102 is a simple and fast solution for screening proteins from various rat tissues. We've streamlined the western blotting procedure by eliminating the sample aquisition, 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™ Rat Tissues IMB-102 contains denatured proteins from rat 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 Sprague Dawley rats. 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™ Rat Tissues IMB-102. Approximately 20 µg per lane of different rat tissue lysate 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.

References:

1. Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidman, J.G., Smith, J., Struhl, K., Current Protocols In Molecular Biology, 1998, V2, 10.8.1-10.8.16.

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)

Human Tissues (Cat. No. IMB-103)

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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