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INSTA-Blot™ Human Cell Lines

Catalog No.: IMB-105

Contents: One INSTA-Blot™

Description: Ready-to-use PVDF membrane with 11 human cell line 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:

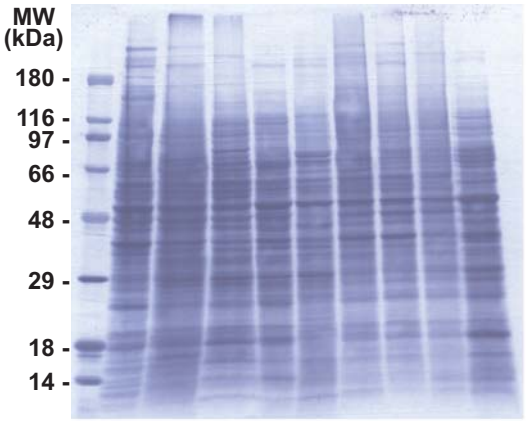
INSTA-Blot™ is a ready-to-use PVDF membrane containing denatured protein from cells or tissue lysates. The INSTA-Blot™ Human Cell Lines IMB-105 is a simple and fast solution for screening proteins from various human cell lines. We've streamlined the western blotting procedure by eliminating the sample 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 Cell Lines IMB-105 contains denatured proteins from human cell line lysates (See Cell Lysate Preparation) 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.

Cell Lysate Preparation:

Human cell lines are lysed in 1X 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 cell 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 Cell Lines IMB-105. Approximately 20 µg per lane of human cell line lysates are resolved by SDS-PAGE, transfered onto PVDF membrane and stained with amido black. Lanes: 1. Molecular weight marker; 2. HeLa; 3. Jurkat; 4. Daudi; 5. HEK 293; 6. Rh30; 7. A375; 8. T98G; 9. HCT-116; 10. Hep-2.

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.

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)

Human Cell Line Lysates

Hela cell line lysate (cervical carcinoma) (Cat. No. 40161)

Jurkat cell line lysate (T cell leukemia) (Cat. No. 40162)

Daudi cell line lysate (lymphoma, Burkitt) (Cat. No. 40163)

HEK293 cell line lysate (embryonic kidney) (Cat. No. 40164)

A549 cell line lysate (lung carcinoma) (Cat. No. 40165)

Rh30 cell line lysate (rhabdomyosarcoma) (Cat. No. 40166)

A375 cell line lysate (amelanotic melanoma cell line) (Cat. No. 40168)

HL60 cell line lysate (promyelocytic cell line) (Cat. No. 40169)

SK-N-SH cell line lysate (human neuroblastoma cell line) (Cat. No. 40170)

MCF-7 cell line lysate (breast cancer derived cells) (Cat. No. 40171)

T98G cell line lysate (human glioma cell line) (Cat. No. 40172)

Hep-2 cell line lysate (human larynx squamous cell carcinoma) (Cat. No. 40173)

HCT-116 cell line lysate (human colon epithelial carcinoma cells) (Cat. No. 40174)

Ramos cell line lysate (human Lymphoblast, Burkitt's lymphoma) (Cat. No. 40175)

INSTA-Blot™

Mouse Tissues (Cat. No. IMB-101)

Rat Tissues (Cat. No. IMB-102)

Human Tissues (Cat. No. IMB-103)

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)

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.

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