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INSTA-Blot™ Human Cell Lines

Catalog No.: NBP2-30114

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:

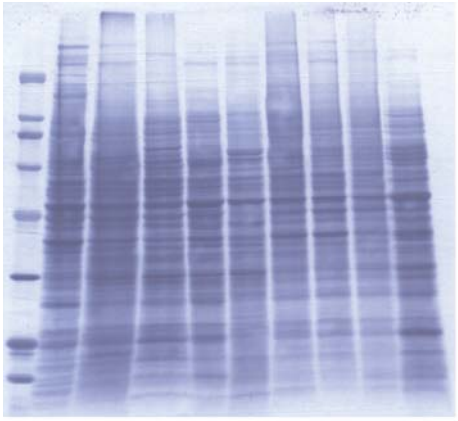
INSTA-Blot™ is a ready-to-use PVDF membrane containing denatured protein from cells or tissue lysates. The INSTA-Blot™ Human Cell Lines NBP2-30114 is a simple and fast solution for screening proteins from various human cell lines. We've stream-lined the western blotting procedure by eliminating the sample aquisition, preparation, SDS-PAGE and electro-blotting steps. With INSTA-Blot™, immunoblotting is an easy six-step proce-dure: blocking, primary antibody incubation, wash, secondary antibody incubation, wash, and develop.

Preparation:

INSTA-Blot™ Human Cell Lines NBP2-30114 contains denatured pro-teins 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 NBP2-30114. 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 (Mark12); 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.

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.

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)

Human Cell Line Lysates

Hela cell line lysate (cervical carcinoma) (Cat. No. NBP2-25045)

Jurkat cell line lysate (T cell leukemia) (Cat. No. NBP2-25046)

Daudi cell line lysate (lymphoma, Burkitt) (Cat. No. NBP2-25047)

HEK293 cell line lysate (embryonic kidney) (Cat. No. NBP2-25048)

A549 cell line lysate (lung carcinoma) (Cat. No. NBP2-25049)

Rh30 cell line lysate (rhabdomyosarcoma) (Cat. No. NBP2-25050)

A375 cell line lysate (amelanotic melanoma cell line)

(Cat. No. NBP2-25051)

HL60 cell line lysate (promyelocytic cell line) (Cat. No. NBP2-25052)

SK-N-SH cell line lysate (human neuroblastoma cell line)

(Cat. No. NBP2-03965)

MCF-7 cell line lysate (breast cancer derived cells)
(Cat. No. NBP2-25053)

T98G cell line lysate (human glioma cell line) (Cat. No. NBP2-25054)

Hep-2 cell line lysate (human larynx squamous
cell carcinoma) (Cat. No. NBP2-25055)

HCT-116 cell line lysate (human colon epithelial carcinoma
cells) (Cat. No. NBP2-25056)

Ramos cell line lysate (human Lymphoblast, Burkitt's
lym-phoma) (Cat. No. NBP2-25057)

INSTA-Blot™

Mouse Tissues (Cat. No. NBP2-30111)

Rat Tissues (Cat. No. NBP2-30112)

Human Tissues (Cat. No. NBP2-30113)

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)

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.