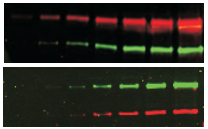
IR-BLOT

MULTIPLEX DETECTION:

- Be sure to use primary antibodies from different host species.
- Incubate the two **IR-BLOT** secondary antibodies together in the same diluted antibody solution.
- IR-Blot 800 secondary antibody recommended for your low-abundant protein to have low background and high sensitivity.
- IR-Blot 700 secondary antibodies recommended for your housekeeping protein or your more abundant protein.



Vinculin -IR-BLOT 700 Goat anti-Mouse
HSP90 -IR-BLOT 800 Goat anti-Rabbit
Vinculin -IR-BLOT 800 Goat anti-Mouse
HSP90 -IR-BLOT 700 Goat anti-Rabbit

Multiplex fluorescent western blotting for the detection of vinculin and HSP90 on two-fold serial dilutions of HeLa whole cell lysates. Imager: ODISSEY®CLX –LI-COR

Related products

Downloads: http://www.cyanagen.com/downloads/product-manuals/

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CYANAGEN
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THE RIGHT LIGHT

IR-BLOT

Secondary Antibodies

FWB

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IR-BLOT

TECHNICAL DESCRIPTION

IR-BLOT secondary antibodies are high quality secondary antibodies optimized for quantitative western blotting in the infrared region.
These antibodies are highly cross-adsorbed, so ensuring extremely low background and very specific signal.The high fluorescence intensity of IR-BLOT secondary antibodies allows a very high sensitivity and an optimal signal to noise ratio, both in the 700 nm and 800 nm channel.
IR-BLOT secondary antibodies can be used on instruments with excitation and emission filters in the infrared region.

STORAGE CONDITIONS

Protect from light. Store at +4°C prior to reconstitution. Reconstitute the content of the vial with 0.1 mL of ultrapure water. Mix gently by inverting and allow to rehydrate for at least 30 minutes before use. Centrifuge the product if particulates are seen in the solution after standing at room temperature. Once reconstituted, the product is stable for up to 3 months at +4°C.

IR-BLOT

FEATURES

High specificity
Cross-adsorbed antibodies for ten species

High sensitivity
High signal intensity and low background

Quantitative
Accurate quantitation in a with wide dynamic range

Multiplex
Optimized for simultaneous detection of different proteins in multicolor IR-western blotting

Extended signal stability
Signal is highly stable: blots can be stored and reimaged without alteration in signal intensity

Reproducible
Fluorescent signal intensity does not vary with time of exposure

IR-BLOT

QUICK START PROTOCOL

Recommended dilution:

Application	Recommended	Suggested Range
Fluorescence Western Blotting	1:25000	1:15000 – 1:50000*

*Optimal dilution will vary and should be determined empirically.

•After membrane transfer, rewet nitrocellulose membrane in PBS and PVDF membranes in methanol and wash with PBS.
•Incubate in Blocking Buffer for 30÷60 min with gentle agitation. During blocking do not use detergents.
•Rinse the membrane twice with TBS-T/PBS-T Buffer.
•Incubate in the primary antibody solution for 1-2 hours at RT.
•Wash 3 times quickly and 4 times for 5 min with TBS-T/PBS-T Buffer with 0.1% Tween®20 and/or 0.005% of SDS with gentle agitation.
•Incubate in the secondary antibody dilution for 30÷60 min at RT in a tray covered with aluminum foil. To reduce background, add detergents to your diluted antibody (Tween®20 from 0.1 to 0.2% and/or 0.01% of SDS).
•Wash 3 times quickly and 4 times for 5 min with TBS-T or PBS-T Buffer with gentle agitation. For better results, add 0.005% of SDS.
•Wash 2 times quickly with PBS or TBS. Your membrane is ready to image.