# IR-BLOT Secondary Antibodies

## for Fluorescent Western Blot

IR-BLOT secondary antibodies are high-quality secondary antibodies optimized for quantitative Western blot in the infrared region. These antibodies are highly cross-adsorbed, so ensuring extremely low background and specific signal. The high fluorescence intensity of IR-BLOT secondary antibodies allows very high sensitivity and an optimal signal-to-noise ratio, both in the 700 nm and 800 nm channel. IR-BLOT secondary antibodies are optimized for multiplex infrared Western blot and lower optimal working dilution. IR-BLOT secondary antibodies can be used on instruments with excitation and emission filters in the infrared region. They are available as goat anti-mouse and goat anti-rabbit.

## Benchmarking

IR-BLOT secondary antibodies exhibit top-level performance for the most challenging Western blot application.

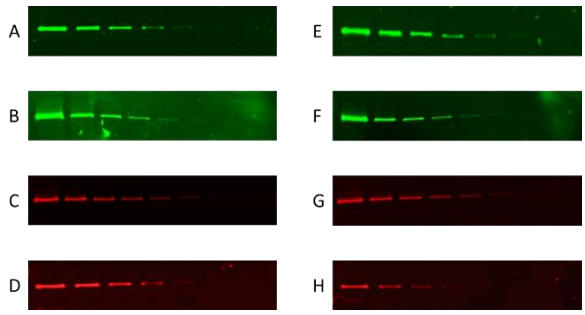


**Figure 1. Fluorescent Western blot with Cyanagen IR-BLOT and LI-COR IRDye® Secondary antibodies.** Sample: 2-fold dilution series of HeLa whole cell lysate (abcam®) from 5µg to 0.078 µg of total protein. Membrane: Trans-Blot® Turbo™ Mini Nitrocellulose Transfer Packs (Bio-Rad). Blocking: 3% ECL™ Blocking Agent (GE Healthcare) in PBS. Primary antibody: Rabbit anti-Human HSP-90 (Santa Cruz Biotechnology) 1:4000 or Mouse anti-Human Vinculin (Sigma) 1:5000. Secondary antibody: A) IR-BLOT 800 Goat anti-Mouse (Cyanagen); B) IR-BLOT 800 Goat anti-Rabbit (Cyanagen); C) IR-BLOT 700 Goat anti-Mouse (Cyanagen); D) IR-BLOT 700 Goat anti-Rabbit (Cyanagen); E) IRDye® 800CW Goat anti-Mouse (LI-COR); F) IRDye® 800CW Goat anti-Rabbit (LI-COR); G) IRDye® 680RD Goat anti-Mouse (LI-COR); H) IRDye® 680RD Goat anti-Rabbit (LI-COR); 1:25000. Imager: ODISSEY ® CLX – LI-COR.

## Increased Quantification Accuracy

Fluorescent Western blot is the best option for quantitative and accurate protein expression analyses. In fluorescent Western Blot, the fluorescence does not depend on an enzymatic reaction, and the signal is directly proportional to the abundance of target protein bound to fluorophore-conjugated antibody. IR-BLOT secondary antibodies offer a wide linear dynamic range with an excellent $R^2$ value > 0.99 over a broad range of protein levels (Figure 2), allowing the user to quantify protein bands accurately.

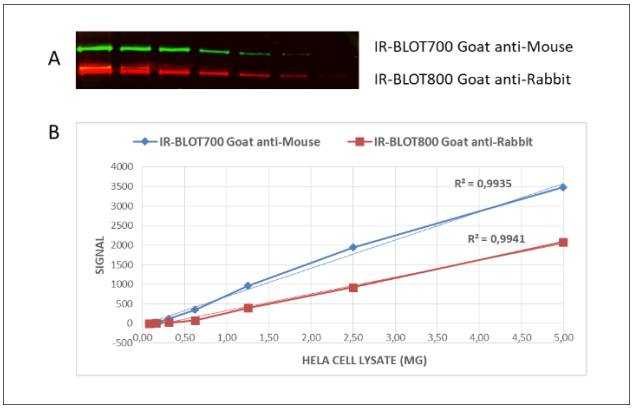


**Figure 2. Enhanced accuracy in Western blot using IR-BLOT Secondary Antibodies.**

A) Western blot detection of vinculin and HSP-90 on HeLa cell lysate with IR-BLOT Secondary antibodies on 2-fold dilutions of HeLa whole cell lysate. Results are the mean of six replicates. Blots were incubated with Rabbit-anti Human HSP-90 (1:4000) and Mouse anti-vinculin (1:5000). Imager: ODISSEY ® CLX – LI-COR.
B) Integrated signal intensity. Data are the mean of six replicates. $R^2$= coefficient of determination.

## High sensitivity

IR-BLOT secondary antibodies' excellent brightness and low background result in a high signal-to-noise ratio comparable to the detection level of the most ECL substrates (Figure 3). These antibodies are ideal for applications requiring very high signal intensity and long signal duration.

**Cyanagen S.R.L.** Via degli Stradelli Guelfi 40/C - 40138 Bologna (BO) - Italy
Tel./ Fax : +39 051 534063 email: info@cyanagen.com- PEC: mail@pec.cyanagen.it
Web: www.cyanagen.com

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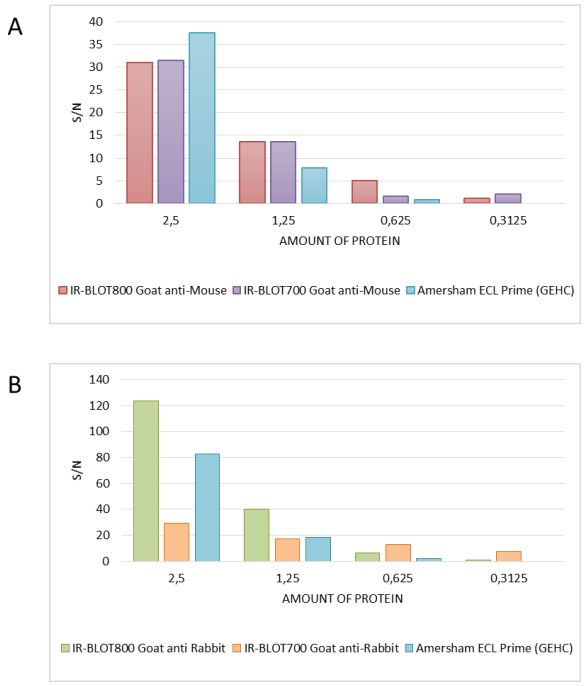


**Figure 3. Low background for high sensitive detection with IR-BLOT secondary antibodies.** Western blot detection of HDAC-1 or vinculin on HeLa cell lysate with mid-level ECL substrates (WESTAR Antares – Cyanagen, Amersham ECL Prime – GE Healthcare) or IR-BLOT secondary antibodies. Blots were incubated with Mouse anti-vinculin 1:4000 (A) or Rabbit-anti Human HDAC-1 1:2500 (B) and Secondary antibody-HRP or IR-BLOT Secondary antibody 1: 100000. Signal-to-noise ratio (S/N) was calculated on at least three replicates.

## Extreme signal duration

Unlike chemiluminescent Western blot, where signal intensity decreases very quickly over time, in fluorescent Western blot signal intensity is highly stable: the blots can be stored and reimaged after weeks without decrease in signal intensity.

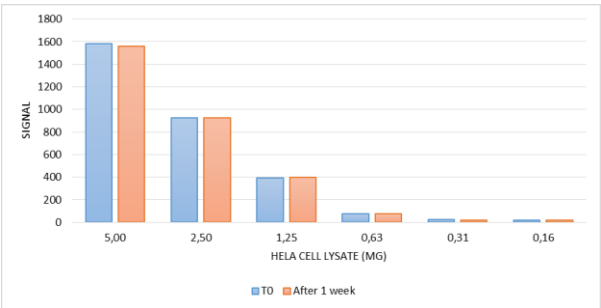


**Figure 4. Signal duration of IR-BLOT over time.** Six replicate of Western blots, containing 2-fold dilution series of HeLa whole cell lysate were incubated with Mouse anti-Vinculin 1:5000 and IR-BLOT 800 Goat anti-Mouse 1:25000 and were simultaneously imaged with Odyssey™ Clx (LI-COR) immediately after the experiment (T0) and after one week with membranes stored at 4°C.

## Multiplexing

Fluorescent Western Blot offers the ability to assay multiple targets on the same blot, at the same time, without stripping and reprobing the blot. Multiplexing is less time consuming and decreases the risk of error from comparing data from different experiments. It allows measuring multiple targets in a single experiment, decreasing needed sample volume. This is crucial when the sample is scarce or precious. IR-BLOT secondary antibodies can be used with other infrared or visible light emitting dye-conjugated antibodies for multiplex imaging applications.

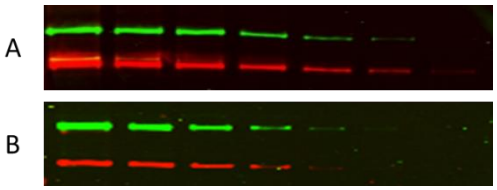


**Figure 5. Multiplex fluorescent western blot for the detection of Vinculin and HSP-90 on HeLa whole cell lysates with Cyanagen IR-BLOT Secondary antibodies.** Sample: 2-fold dilution series of HeLa whole cell lysate (abcam®) from 5µg to 0.078 µg of total protein. Membrane: Trans-Blot® Turbo™ Mini Nitrocellulose Transfer Packs (Bio-Rad). Blocking: 3% ECL™ Blocking Agent (GE Healthcare) in PBS. Primary antibody: Rabbit anti-Human HSP-90 (Santa Cruz Biotechnology) 1:4000 and Mouse anti-Human Vinculin (Sigma) 1:5000. Secondary antibody: A) IR-BLOT 700 Goat anti-Mouse (red) and IR-BLOT 800 Goat anti-Rabbit (green); B) IR-BLOT 700 Goat anti-Rabbit (red) and IR-BLOT 800 Goat anti- Mouse (green)1:25000. Imager: ODISSEY ® CLX – LI-COR.

## Conclusions

IR-BLOT secondary antibodies are Cyanagen highly cross-adsorbed antibodies optimized for fluorescent western blot. Their excellent signal stability, high reproducibility and wide linear dynamic range make IR-BLOT secondary antibodies the perfect solution for an accurate quantification of western blot results.

| | Fluorescent Detection | Advantages |
|---|---|---|
| **Multiplexing** | Yes | Save samples, save time, comparative studies in the same blot |
| **Sensitivity** | High | High sensitivity for every applications |
| **Stability** | Weeks to Months | Re-imaging after weeks, high reproducibility |
| **Detection** | Direct, quantitative method | Accurate quantitation with a wide linear dynamic range |

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Web: www.cyanagen.com