Western blotting reagents

Amersham™ CyDye™ 700 and 800

Amersham CyDye 700 and 800 secondary antibodies are labeled with near-infrared (NIR) fluorophores that emit light at wavelengths of 700 nm or 800 nm. By using Amersham CyDye 700 and 800 secondary antibodies together with a pair of appropriate rabbit or mouse primary antibodies, you can perform multiplexed experiments.

Fig 1. Amersham CyDye 700 and 800 NIR-labeled secondary antibodies for fluorescent Western blotting.

Key benefits

- Easy handling and a strong signal-to-noise ratio yield reproducible results
- Multiplexing analysis of more than one target protein on a single blot increases process efficiency
- Total protein normalization (TPN) generates reliable and accurate quantitation
- Stable signals allow membranes to be stored and rescanned for future studies

Fluorescent Western blotting

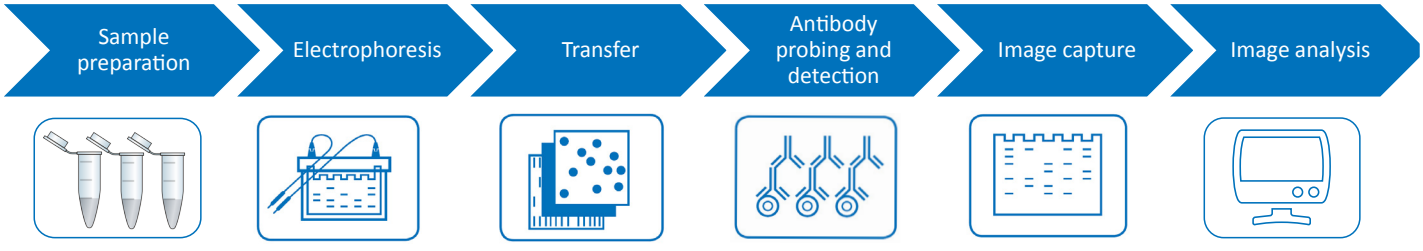


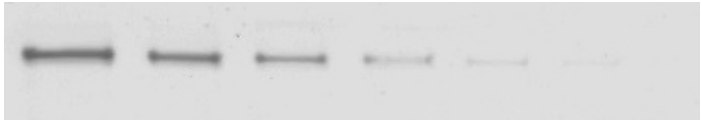
Fig 2. Fluorescent Western blotting workflow, showing the steps required to obtain quantitative data.

Fluorescent Western blotting delivers quantitative and consistent results and involves multiple steps and resources (Fig 2). Apart from the primary antibodies which are specific to each target protein, a common setup of equipment and reagents can be used for all Western blotting. GE Healthcare provides key items to complete this workflow, including Amersham CyDye 700 and 800 secondary antibodies. These antibodies have been tested to make sure reliable results are obtained from fluorescent Western blots.

Compared to other Western blotting detection technologies, fluorescent Western blotting yields stable signals that do not fade over time. Results remain consistent between blots and exposures. Multiplex NIR fluorescent labels provide accurate identification and quantitation of proteins with similar molecular weights, which is useful for studying protein phosphorylation. Fluorescent Western blotting also allows simultaneous detection of target and housekeeping proteins, which enables you to normalize data to an internal standard without adding steps for stripping and reprobing. This Western blotting detection technology uses simplified steps and yields accurate results.

Amersham CyDye 700 and 800 secondary antibodies in combination with mouse and rabbit primary antibodies (Fig 3) deliver an excellent signal-to-noise ratio. The dyes enable the detection of two proteins in the same blot with a prelabeled sample and 3-channel scanner. This multiplexing analysis can be combined with TPN. Note that nitrocellulose membranes are recommended because they give a low and even background. The use of polyvinylidene difluoride (PVDF) membranes may require additional optimization of the blocking and probing solution.

(A)



(B)

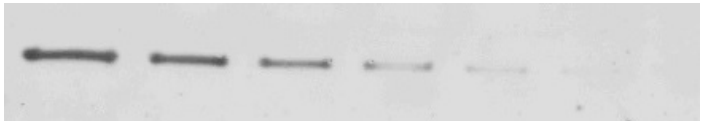
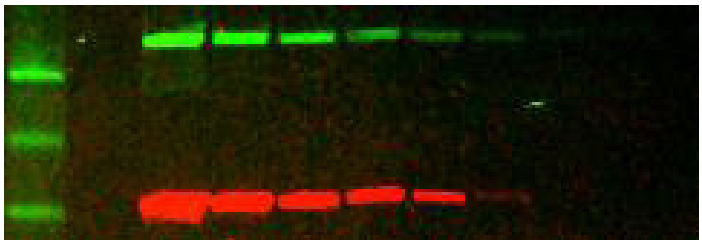


Fig 3. Fluorescent Western blotting detection of (A) vinculin using Amersham CyDye 800 goat anti-mouse, and (B) HSP90 Amersham CyDye 700 goat anti-rabbit. Two-fold dilution of Hela cell whole lysate starting from 5 µg were loaded in each lane.

Multiplex experiments and TPN

Using the Amersham CyDye 700 and 800 secondary antibodies together enables detection of two different target proteins in the same blot (Fig 4).

(A)



(B)

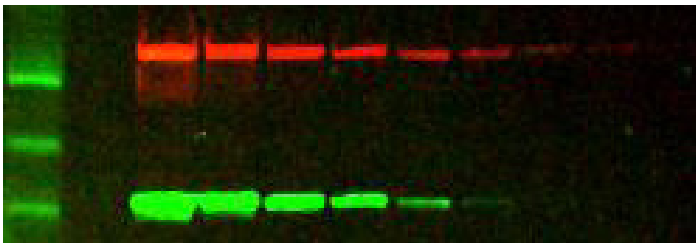


Fig 4. Multiplex detection of ß-galactosidase and tubulin with images captured by Amersham Typhoon 5. Primary antibodies were diluted 1:5000 and secondary antibodies 1:20 000. (A) ß-galactosidase in green (Amersham CyDye 700 goat anti-rabbit), 48 ng diluted 1:2 to 0.19 ng, and tubulin in red (Amersham CyDye 800 goat anti-mouse), 25 ng diluted 1:2 to 0.10 ng; (B) Secondary antibodies reversed from (A).

Figure 5 shows a Western blot for which the applied sample volumes were intentionally varied to demonstrate the power of TPN. Normalization compensates for loading variation and variations during transfer, but not for uneven probing. The traditional approach to normalization involves the use of house-keeping proteins, however this can lead to unreliable results due to environmental effects on protein expression. Prelabeling of the sample with Cy™3 enables detection of two different target proteins and the use of TPN, the preferred normalization method (1). TPN uses the total sample signal in the lane to normalize the target protein signals. This is a more robust and reliable way of normalization than the use of a signal from a house-keeping protein (2).

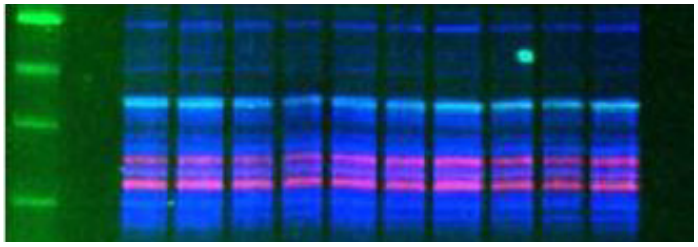


Fig 5. Triplex detection with prelabeled sample. Images captured by Amersham Typhoon 5. This experiment requires either Amersham Typhoon 5 or Amersham Typhoon NIR Plus to detect Cy3 signal. Primary antibodies were diluted 1:2500 and secondary antibodies 1:20 000. Prelabeled CHO-cells 8 to 12 µg (Cy3, dark blue) and detection of ERK (Amersham CyDye 700 goat anti-rabbit, red) and tubulin (Amersham CyDye 800 goat anti-mouse, light blue).

Figure 6 compares the non-normalized ERK signal with the ERK signal normalized against the total signal in each lane. A house-keeping gene product normalization was also performed using the tubulin signal in each lane. The coefficient of variation (CV) for the ERK signal was calculated for all normalization strategies (Table 1). The CV was lowest using TPN, demonstrating the advantage of this approach for normalization of Western blots.

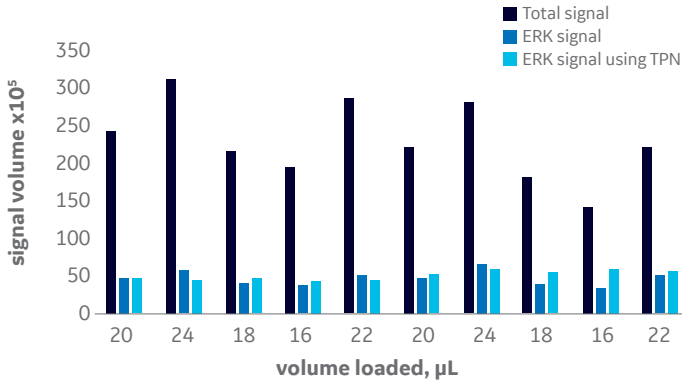


Fig 6. Total signal, ERK signal and ERK signal normalized against total signal volume. All quantitation and calculations were performed by ImageQuant™ TL image analysis software.

Table 1. Coefficient of variation (CV) values for the ERK signal using different normalization methods.

	Non-normalized	Tubulin normalized	TPN
ERK CV	21.1	19.5	11.6

Ordering information

System	Quantity	Product code
Amersham CyDye 700 goat-anti-mouse (0.1 mg)	1	29360784
Amersham CyDye 700 goat-anti-mouse (0.5 mg)	1	29360785
Amersham CyDye 700 goat-anti-rabbit (0.1 mg)	1	29360786
Amersham CyDye 700 goat-anti-rabbit (0.5 mg)	1	29360787
Amersham CyDye 800 goat-anti-mouse (0.1 mg)	1	29360788
Amersham CyDye 800 goat-anti-mouse (0.5 mg)	1	29360789
Amersham CyDye 800 goat-anti-rabbit (0.1 mg)	1	29360790
Amersham CyDye 800 goat-anti-rabbit (0.5 mg)	1	29360791

Related products

Product	Quantity	Product code
Sample preparation		
PD MiniTrap™ G-25	50 columns	28918007
Mammalian Protein Extraction Buffer	500 mL	28941279
Protein markers		
Amersham ECL Plex™ Fluorescent Rainbow™ Markers	120 µL	RPN850E
Amersham ECL Plex Fluorescent Rainbow Markers	500 µL	RPN851E
Blotting membranes		
Amersham Protran™ Premium 0.45µm NC 80mm × 90 mm	25 sheets/ PK	10600096
Amersham Protran Premium Sandwich 0.45µm NC + 3MM Chr Paper 80mm × 90mm	10+20/PK	10600117
Blotting paper		
3MM Chr	100 sheets	3030-861

Product	Quantity	Product code
Blocking agent		
Amersham ECL Prime Blocking Reagent	40 g	RPN418
Imagers		
Amersham Typhoon 5	1	29187191
Amersham Typhoon NIR Plus	1	29264463
Amersham Typhoon NIR	1	29238583
Image analysis software		
ImageQuant TL, node locked	1	29291744
ImageQuant TL Security, node locked	1	29291745

Related literature

Literature name
Data file: Amersham Typhoon Biomolecular Imager
Handbook: Imaging, principles and methods
Handbook: Western blotting, principles and methods
Protocol: Western blotting with Amersham QuickStain

References

1. Western blotting with *Amersham QuickStain*, GE Healthcare, KA3227010618FL, (2018).
2. Bjerneld, E. *et al.* Pre-labeling of diverse protein samples with a fixed amount of Cy5 for sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis. *Anal. Biochem.* **484**, 51–57 (2015).

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