Electrophoresis reagents

Amersham™ QuickStain

The Amersham QuickStain Kit contains a Cy™5 fluorophore and labeling buffer for easy detection of proteins in SDS-PAGE gels and on Western blotting membranes. The ready-to-use Cy5 N-hydroxysuccinimide (NHS) ester and Tris labeling buffer ensure robust and consistent labeling for detection of proteins in diverse samples.

Key benefits

- **Ease of use:** Ready-to-use reagents for fast and convenient analysis of proteins following one-dimensional (1-D) electrophoresis and/or Western blotting
- **Fast protocols:** Choose between a quick 5 min protocol and a standard 30 min quantitative protocol
- **Wide sample range:** Stain complex and purified samples with protein concentrations ranging from 1 µg/ml to 20 mg/ml, without the need to pre-measure protein concentration
- **Sensitivity:** As good as silver staining, sub-ng sensitivity
- **Broad dynamic range:** With a fluorescence imager or scanner, it is possible to simultaneously detect main bands and impurity levels down to parts per thousand (0.1%)
- **Robustness:** A 10-fold dilution of samples with the labeling buffer, which has a high buffering capacity, ensures reproducible labeling at optimal pH for a wide variety of samples
- **Versatility:** Most materials exhibit low Cy5 fluorescence background, which makes the kit compatible with many common gel formats and membranes

With the easy Cy5 prelabeling of Amersham QuickStain, there is no need to do post-staining (e.g., Coomassie™ or silver staining) of gels or Ponceau S staining of membranes (see Fig 1). The kit is compatible with common gel-buffer electrophoresis systems, including Tris-acetate and Tris-glycine buffer systems. Many plastic materials exhibit a low fluorescence background in the Cy5 spectral region, which

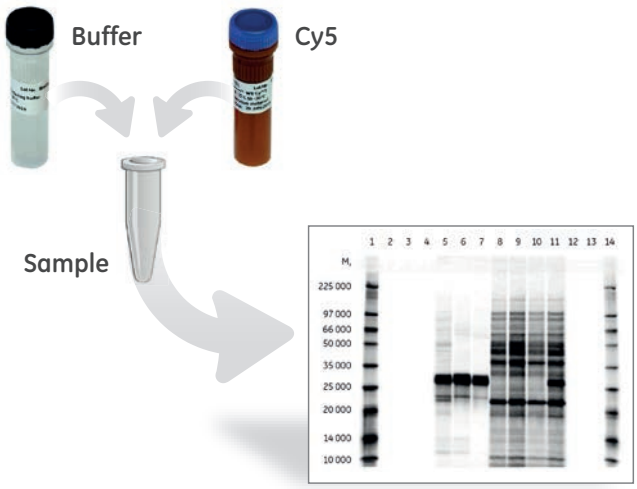

Fig 1. Amersham QuickStain is designed for easy detection of proteins after SDS-PAGE and/or after Western blotting. Simply add the provided labeling buffer and Cy5 to the sample prior to electrophoresis.

makes Cy5 staining well suited for precise quantitation of proteins using a wide variety of gel formats (i.e., precast, homecast, cassettes, backed, and nonbacked). Using CCD imagers and laser scanners from GE Healthcare's Life Sciences, proteins can be detected with high sensitivity and a broad linear dynamic range. As a result, Amersham QuickStain can be used for a wide variety of protein analysis applications, from quick visualization of protein content in different samples to accurate quantitation of target proteins using calibration curves.

Traditional staining methods often limit the dynamic range, typically to one or two orders of magnitude for silver and Coomassie staining. In contrast, fluorescence detection has a much broader detection window. The protocols used with the Amersham QuickStain kit have been optimized to take advantage of this broad dynamic range. As shown in Figure 2, Amersham QuickStain gives substantially better signal-to-noise and signal-to-background ratios compared with Coomassie staining.

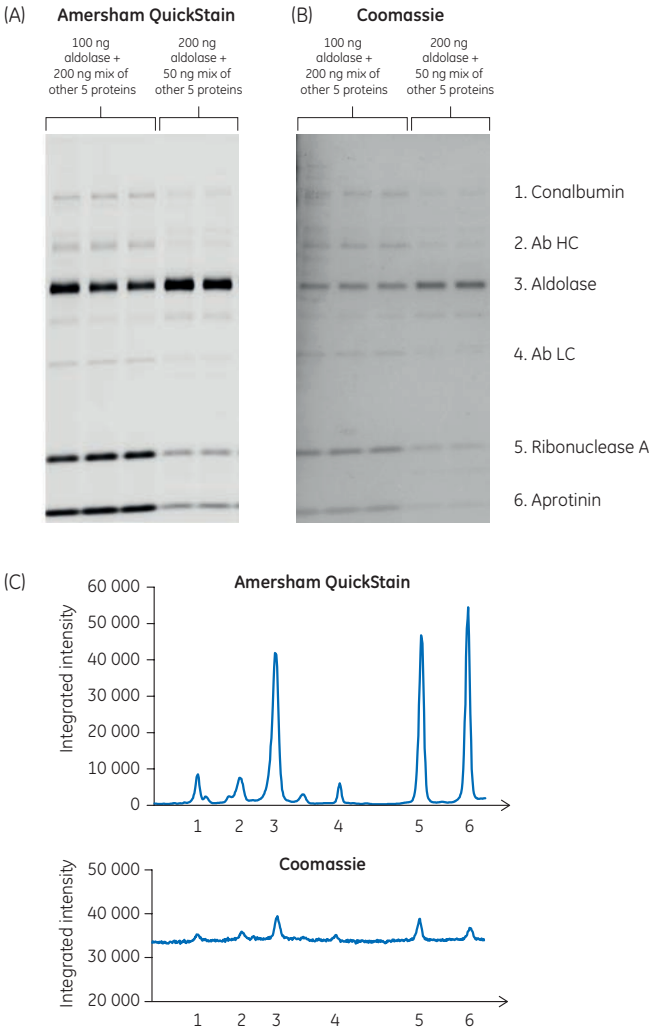

Fig 2. Comparison of gel images and lane profiles using (A) Amersham QuickStain and (B) Coomassie staining. The two gels were loaded with the same quantities of six proteins per well, as indicated above each lane. Novex™Tris-glycine gels were used, and gel images were generated using Typhoon™ FLA 9500 scanner (QuickStain) and ImageQuant™ LAS 500 imager (Coomassie). The lane profiles in (C) show the intensity of bands in lane 3 for each image.

Labeling protocols for SDS-PAGE

The Amersham QuickStain kit is based on Cy5 NHS ester labeling of reactive amino groups in proteins. To ensure minimal interference from sample components and to maintain an optimal pH for labeling, the prelabeling protocol starts with a 10-fold dilution of the sample using the Amersham QuickStain labeling buffer. There is no need to remove excess dye, because free dye migrates faster than the proteins of interest and therefore ends up at the bottom of the gel. The compositions of the two kit components are provided in Table 1.

Table 1. Amersham QuickStain components

Cy5	Cy5 NHS ester in anhydrous dimethyl sulfoxide (DMSO)
Labeling Buffer	Tris-HCl labeling buffer with 0.1% sodium dodecyl sulfate (SDS), pH 8.7 at 25°C

Quick protocol – for qualitative analysis

1. Dilute sample 10-fold with labeling buffer
2. Add Cy5 to the sample
3. Heat sample at 95°C for 3–5 min
4. Add sample loading buffer containing lysine*
5. Heat sample at 95°C for 3 min
6. Load sample on gel

Total time less than 15 min

Quantitative protocol

1. Dilute sample 10-fold with labeling buffer
2. Add Cy5 to the sample
3. Incubate sample for 30 min at room temperature
4. Add sample loading buffer containing lysine*
5. Heat sample at 95°C for 3 min
6. Load sample on gel

Total time less than 40 min

*Lysine quenches the labeling reaction. Add DTT to loading buffer if running reducing SDS-PAGE.

Note: In Western blotting applications we recommend performing the labeling of cell lysates in the sample lysis buffer. Doing so will lead to a lower degree of labeling and a lower signal that is suitable for detection on membranes.

Fixed amount of dye in the labeling reaction

Keeping the amount of dye fixed in the labeling reaction and varying the protein concentration (Fig 3) leads to linear dose-response curves and well-resolved protein bands on the gel (1). For accurate quantitation, label calibrants under conditions identical to those used for the samples, and make a calibration curve.

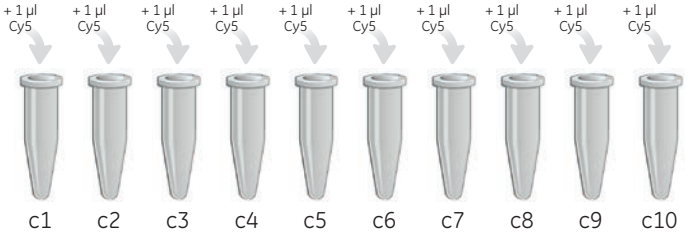

Fig 3. The Amersham QuickStain protocols are based on adding a fixed amount of dye to samples with different protein concentrations. For a 20 µl reaction volume, add 1 µl of Cy5.

GE imagers and scanners for detection of Amersham QuickStain

Both CCD imagers and laser scanners can be used to detect and quantitate signals from Cy5 labeled proteins. Amersham Imager 600RGB (Fig 4, Fig 5) is an advanced CCD imager that can be used to detect three different fluorophores in multiplexing experiments. Amersham Typhoon RGB and 5 systems (Fig 4) are high-end variable-mode laser scanners for the most demanding applications. Both Amersham Typhoon systems have a large working area and many laser-filter combinations. All three instruments are well suited for detection of proteins prelabeled using Amersham QuickStain in gels and on membranes. The high-end detectors (CCDs and photomultiplier tubes) and the 16-bit depth file format enable a linear dynamic range of up to five orders of magnitude. This wide linear range makes it possible to do quantitative measurements of both strong and weak signals in the same image, which is a key benefit for many applications (e.g., purity analysis).

Fig 4. GE's Amersham Imager 600RGB (left) and Amersham Typhoon scanner (right) can be used in Cy5 imaging mode to detect proteins prelabeled using Amersham QuickStain.

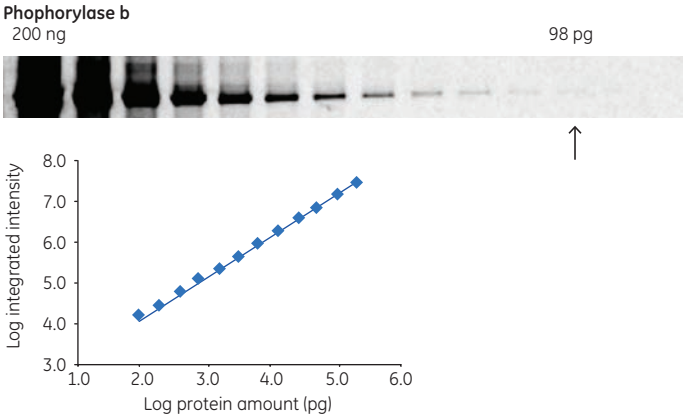

Fig 5. Linearity, dynamic range, and limit of detection for proteins using Amersham QuickStain with Amersham Imager 600RGB. A 2-fold dilution series of Cy5 labeled phosphorylase b sample shows a linear dynamic range of 3.3 orders of magnitude and a detection limit of 100 pg.

Application examples

Amersham QuickStain can be used to detect proteins in different types of SDS-PAGE analysis, including:

- Monitoring of bioprocessing steps, including protein quantitation with calibrants and standard curves
- Purity analysis and fraction screening in protein purification processes
- Detection of proteins on Western blot membranes for comparison to antibody-based detection of target protein

Quantitation of antibodies

Antibodies play a key role in biotechnology and therapeutics. It is important to measure the amount of antibody in a sample and to analyze its purity. Amersham QuickStain was used to detect antibody expressed in Chinese hamster ovary (CHO) cells over time. Samples were labeled with Cy5 prior to SDS-PAGE. Both non-reduced and reduced samples were run on the gels to check the integrity of the antibodies. It was possible to accurately measure the amount of antibody in the samples, using a calibration curve, and to evaluate the purity (Fig 6). The quantities of antibody determined from the SDS-PAGE analysis agreed well with enzyme-linked immunosorbent assay (ELISA) measurements. In this way the production process of an antibody was monitored (2).

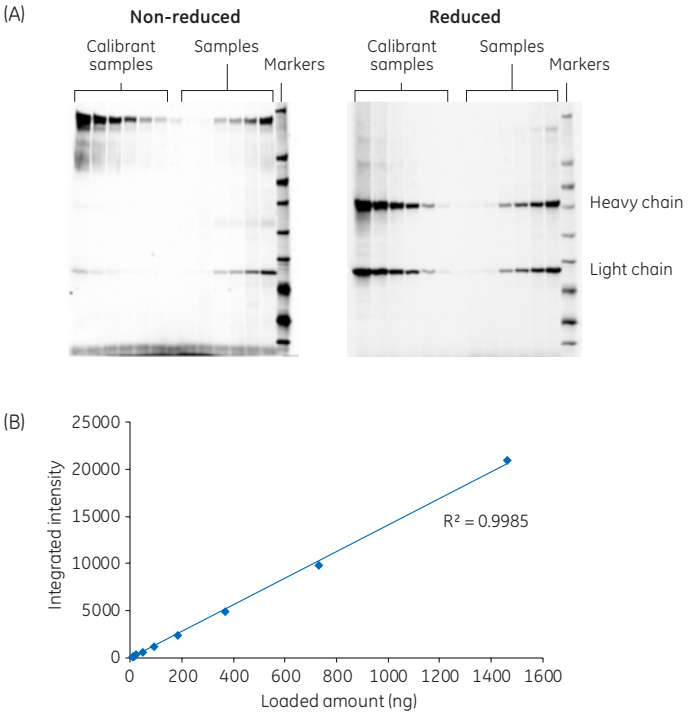

Fig. 6. Detection of antibody in CHO cells; protein was prelabeled using Amersham QuickStain. (A) Images of SDS-PAGE gels. Calibrant samples, samples collected at different times from the cell culture, and markers were loaded onto each gel. The non-reduced gel shows bands of intact antibody, as expected. Reduced samples exhibit the heavy chain band at approximately M_r 50 000 and the light chain band at approximately M_r 23 100. (B) Example of a dose-response curve for a therapeutic monoclonal antibody from a "reduced" gel image similar to the one in the top panel. This curve is for the heavy chain of trastuzumab.

Purity analysis

It is standard practice to use SDS-PAGE to measure the purity of different fractions during protein purifications. The results are used to evaluate the purification process and form the basis for fraction picking and selection of a purification strategy. Thus, it is important to assess both purity and total amount of protein in each fraction. The excellent sensitivity of Amersham QuickStain reveals purity with details that are not seen with Coomassie staining. In fact, signals from impurities as low as 0.1% of the main band's signal intensity can be detected (see Fig 7). The detection limit is similar to the limit for silver staining, but the wide dynamic range allows for quantitation of both weak and strong signals and a more stringent determination of purity. In addition, with SDS-PAGE slab gel analysis of multiple samples loaded in adjacent lanes, it is easy to assign well-resolved bands to protein impurities. In contrast, lane profiles in chip- and capillary-based electrophoresis are more difficult to evaluate.

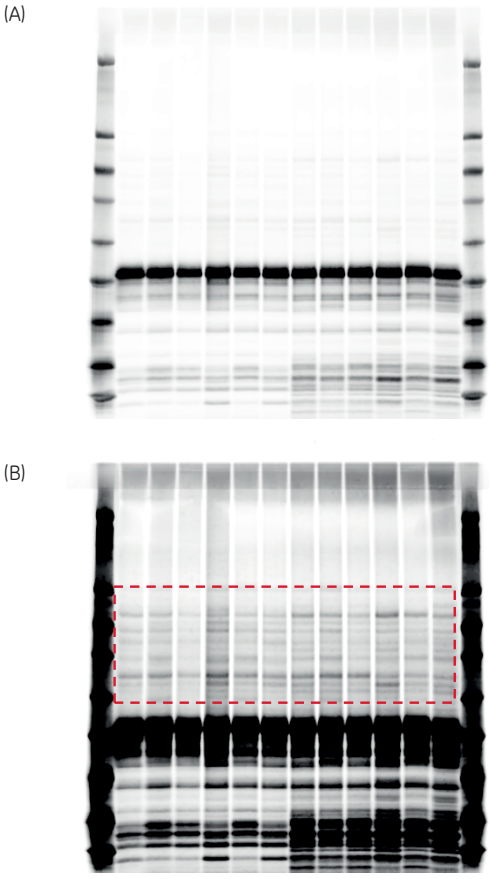

Fig 7. Gel images showing Amersham QuickStain results of different fractions from purification of an MBP-His dual-tagged protein using different immobilized affinity chromatography (IMAC) media. Images A and B are the same image with different contrast settings. Marker samples are loaded in the outer lanes. The signals from highlighted impurity bands (red box) fall within 0.1%–0.7% of the main band's signal intensity.

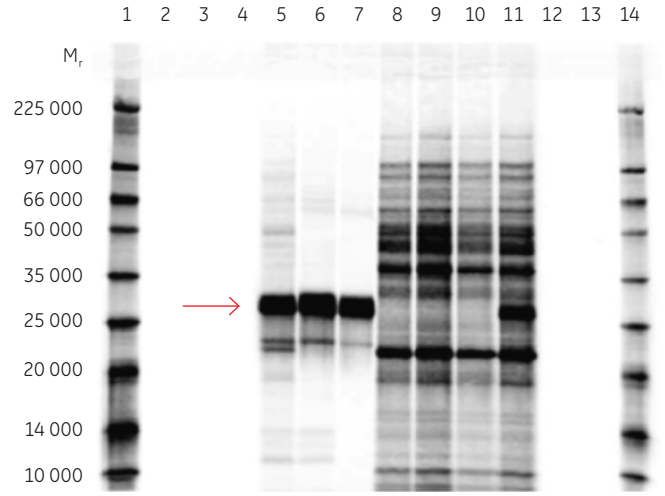

Fig 8. SDS-PAGE analysis of the recovery and purity of his-tagged GFP in the elution pool after a Capto™ Chelating IMAC step using different imidazole concentrations in the column equilibration and wash buffers. Red arrow indicates position of full-length his-GFP. Buffer exchange prior to labeling was performed using the Amersham WB MiniTrap™ Kit. Prior to electrophoresis, Cy5 labeling was performed using Amersham QuickStain.

In the example in Figure 8, the purity of histidine-tagged (his-tagged) GFP in different eluates was analyzed to optimize immobilized metal ion affinity chromatography (IMAC) elution conditions. Amersham QuickStain was used to analyze both the purity and the amount of protein in the different fractions.

Total protein detection on Western blotting membranes

Amersham QuickStain can also replace Ponceau S staining of proteins on membranes. Multiplexing is possible using the multichannel detection capabilities of many imagers and scanners. For example, a Cy3 labeled secondary antibody can be used to detect the target protein of interest, and Amersham QuickStain can be used to measure the total protein content of a sample (Fig 9). This multiplexing capability provides a way to evaluate the transfer efficiency and to adjust for loading errors using normalization (3). It is also possible to perform chemiluminescence Western blotting experiments and compare these results with the total protein content measured using Amersham QuickStain and a suitable CCD imager or scanner.

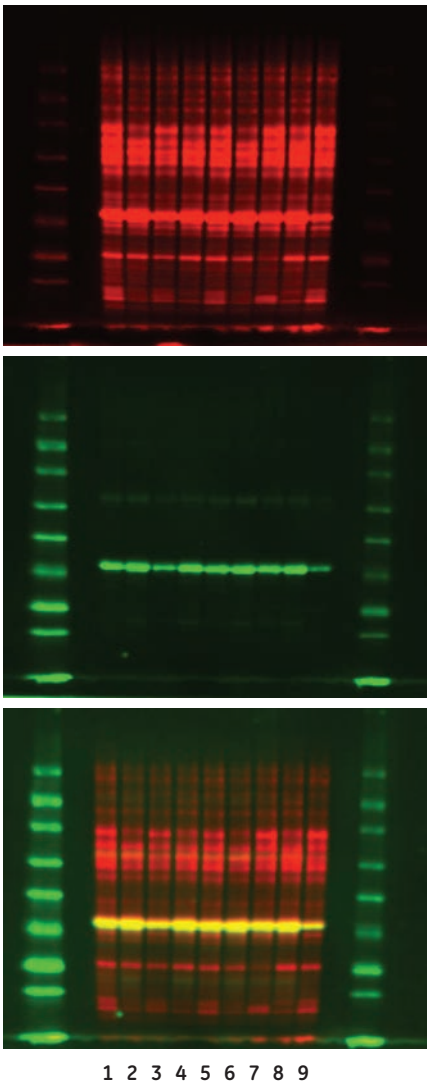

Fig 9. Multiplex detection of total protein and target protein with Amersham QuickStain (Cy5), Amersham ECL Plex™ (Ab-Cy3), and Amersham Imager 600RGB. DHFR (Cy3, green) was detected in nine different samples from a growth optimization of *E coli*. Total protein in the samples was prelabeled with Cy5 (red). The overlay image shows the DHFR band in yellow. Membrane blocking was performed with 3% BSA in PBS-Tween™; rabbit anti-DHFR C-terminal primary antibody was diluted 1:1000; ECL Plex Goat anti-rabbit-Cy3 secondary antibody was diluted 1:2500.

Data storage and image analysis

Data are stored either in linear 16-bit grayscale TIFF file format when using a CCD camera (.tif files) or in square root-encoded 16-bit TIFF file formats when using a laser scanner (.gel files). For image analysis we recommend either Amersham Imager 600 analysis software or ImageQuant TL software. ImageQuant TL is general-purpose software that can be used to analyze 1-D electrophoresis gels, membranes, dot blots, and arrays.

References

1. 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).
2. Application note: Analysis of therapeutic antibodies using Amersham WB system, GE Healthcare, 29114027, Edition AA (2014).
3. Hagner-McWhirter, Å. et *al.* Cy5 total protein normalization in Western blot analysis. *Anal. Biochem.* **486**, 54–61 (2015).

Ordering information

Product	Quantity	Product code
Amersham QuickStain	1	RPN4000

Related products	Quantity	Product code
Sample preparation		
PD MiniTrap G-25	50 columns	28918007
Mammalian Protein Extraction Buffer	500 ml	28941279
Protein G Mag Sepharose™	1 × 500 µl	28444008
Streptavidin Mag Sepharose	2 × 1 ml	28985738
CyDye™ conjugated antibodies		
Amersham ECL Plex goat-α-mouse IgG-Cy3, 150 µg	150 µg	PA43009
Amersham ECL Plex goat-α-rabbit IgG-Cy3, 150 µg	150 µg	28901106
Protein markers		
Amersham ECL Plex Fluorescent Rainbow Markers	120 µl	RPN850E
Amersham ECL Plex Fluorescent Rainbow Markers	500 µl	RPN851E
Blotting paper		
3MM Chr	100 sheets	3030-861
Blotting membranes		
Amersham Hybond™ LFP	1 roll/PK	10600022
Amersham Hybond LFP, 30 × 60 cm	5 sheets /PK	10600040
Amersham Hybond LFP, 20 × 20 cm	10 sheets/PK	10600060
Amersham Hybond LFP, 10 × 10 cm	10 sheets/PK	10600091
Amersham Hybond LFP Sandwich 0.2 PVDF, 8 × 9 cm	For 10 minigel blots	10600123
Amersham Protran™ Premium 0.2 NC	1 roll/PK	10600004
Amersham Protran Premium 0.2 NC, 30 × 60 cm	5 sheets/PK	10600035
Amersham Protran Premium 0.2 NC, 20 × 20 cm	10 sheets/PK	10600050
Amersham Protran Premium 0.2 NC, 10 × 10 cm	10 sheets/PK	10600081
Amersham Protran Premium Sandwich 0.2 NC, 8 × 9 cm	For 10 minigel blots	10600118
Blocking agent		
Amersham ECL™ Prime Blocking Reagent	40 g	RPN418

Related products	Quantity	Product code
Imagers		
Amersham Typhoon 5	1	29187191
Amersham Typhoon RGB	1	29187193
Amersham Imager 600RGB	1	29083467

Related literature	Product code
Data file: Amersham Imager 600	29098107
Data file: Amersham Typhoon Biomolecular Imager	29229537
Handbook: Imaging, principles and methods	29020301
Handbook: Western blotting, principles and methods	28999897
Application note: Accurate comparability assessment of a biosimilar interferon in process development	29115478
Application note: Analysis of therapeutic antibodies using Amersham WB system	29114027

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