Amersham™ WB system

The Amersham WB system is a fully integrated system for SDS-PAGE and Western blotting of proteins based on fluorescence detection. Every stage of the Western blotting process—electrophoresis, scanning, transfer, and probing—is standardized and monitored. Amersham WB system is designed to deliver consistent, quantifiable data from every sample, every time. Reagents for prelabeling and secondary antibodies as well as Amersham WB gel card and membrane (PVDF) card consumables provide reproducibility and convenience in your Western blotting workflow (Fig 1).

Key features and benefits:

- Integrated Western blotting system with standardized protocols in every step minimizes assay variability.
- Dedicated consumables and reagents ensure high quality and reproducibility.
- Optimized prelabeling of samples with Cy™5 NHS chemistry for proven performance without gel staining.
- Reliable normalization by multiplexing target and control fluorescent signals on the same blot.
- Western blot workflow achieved in less than 4 h ensures fast, conclusive data.
- Smart control and evaluation software gives you complete control.

The Amersham WB system consists of the Amersham WB analyzer, Amersham WB software, and dedicated consumables for electrophoresis and Western blotting.

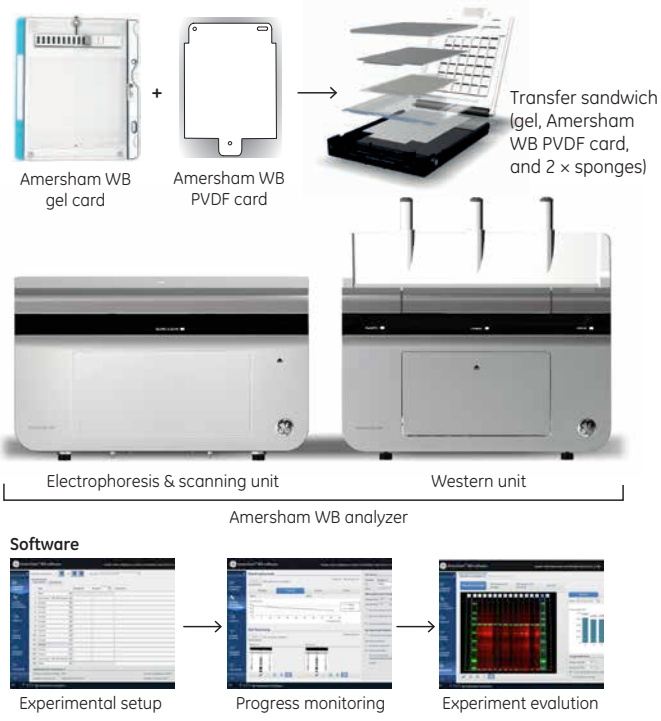


Fig 1. The Amersham WB system for protein analysis provides standardized, quantitative, and reproducible analysis of proteins in your samples in a significantly shorter time compared with traditional workflows/procedures.

Amersham WB analyzer

The Amersham WB analyzer is divided into two instrument units—one for electrophoresis and scanning and one for the full Western blot workflow including transfer, probing, and drying (Fig 2). The electrophoresis & scanning unit is used to separate SDS-treated sample proteins in a polyacrylamide gel. It is also used to scan gels after electrophoresis and polyvinylidene (PVDF) cards after probing and drying in a Western experiment.

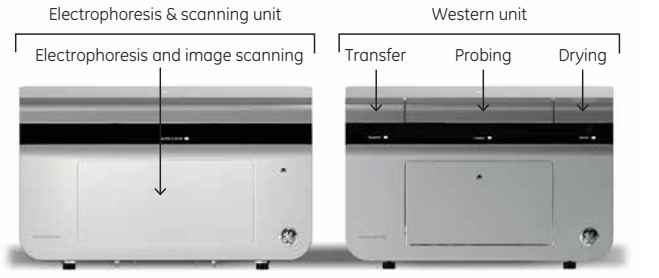


Fig 2. The instrument units and workflow steps of the Amersham WB analyzer.

The Western unit is used to: (1) transfer separated proteins in the gel to the PVDF membrane; (2) probe proteins on the membrane with primary antibodies and secondary antibodies conjugated to a CyDye™; (3) dry the PVDF cards (before scanning in the electrophoresis & scanning unit).

Amersham WB software

Standardized experimental design options with protocols

The system supports many applications from quick screening of protein composition or abundance to advanced quantitative analysis for comparisons of protein levels between samples (Fig 3).

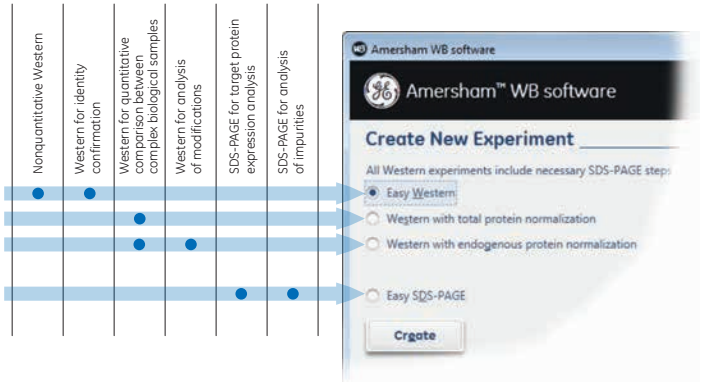


Fig 3. The Amersham WB software guides you to the appropriate application and offers default protocols, detection settings, and evaluation for each step.

Easy control of every step

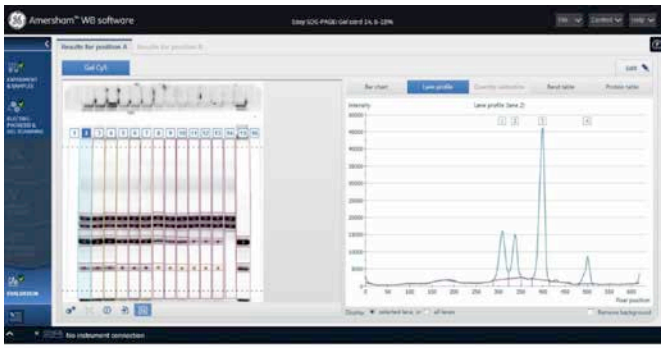
Easy-to-use software for controlling the different steps of the workflow and for automated or manual evaluation of results is part of the system (Fig 4). The instrument control showing ongoing activity and preliminary results is displayed on the left half of the screen. The default input parameters for each protocol can be adjusted if needed on the right side of the control screen.

Fig 4. The Amersham WB software workflow guides you through control screens for each step. All steps have optimized default protocols for convenience. Here, screens for experimental setup, electrophoresis and scanning, as well as probing are shown as examples.

Automated data analysis

When a run is finished, a result is automatically scanned and evaluated. Depending on the application chosen, a suitable view of the data is displayed (Fig 5). It is easy to switch between different view options for further data analysis.

(A)



(B)

(C)

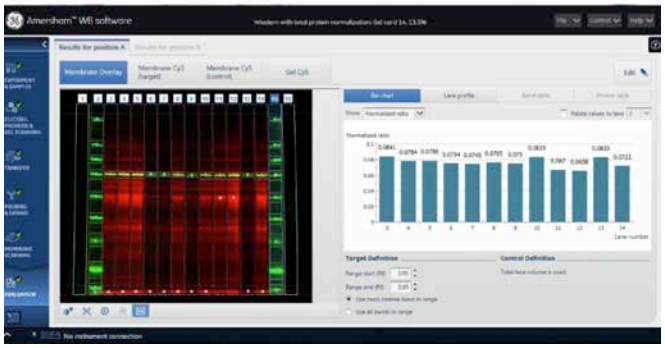


Fig 5. (A) Results view showing the profile of a selected lane. The gel or membrane image is always displayed to the left. (B) Results view showing a calibration curve for quantitation. (C) Results view for normalization using total protein. A bar graph with the normalized amounts is displayed together with an overlay image of the fluorescent signals.

Dedicated, ready-to-use consumables

Amersham WB Cy5 labeling kit for convenient prelabeling

Proteins can be prelabeled with Cy5 and can therefore directly be detected in the gel after electrophoresis (Fig 6).

Labeling is performed easily using the convenient Amersham WB Cy5 labeling kit, which offers the following benefits:

- Optimized Cy5 NHS labeling chemistry for proven performance without prior protein quantitation of sample.
- Ready-to-use Cy5 dye designed for sensitive and broad detection range without quenching effects.
- Covalent conjugation to proteins; no need for post-staining and labeled protein transfers to Western blotting membranes.
- Labeling buffer provided for ease of use; loading buffer without reductant enables native gel electrophoresis.
- Loading buffer with Orange G tracking dye provided for minimal fluorescence background.
- Ready-to-use fluorescent molecular weight markers included for optimal calibration in both Cy3 and Cy5 channels (Fig 7).

In Western experiments, unlabeled or Cy5 prelabeled protein samples are transferred to membranes and target proteins can be detected using Cy3- or Cy5-labeled secondary antibodies. The fluorescence-based detection gives high sensitivity and broad dynamic range. It also enables multiplex analysis on the same Western blot membrane and normalization of target signals to correct for loading errors using endogenous control signal or Cy5 total protein signal. Further, you can simply analyze target signals for total protein.

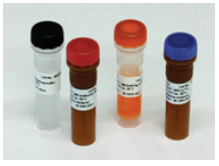


Fig 6. The Amersham WB Cy5 labeling kit consists of color-coded tubes containing ready-to-use reagents for ease of use.

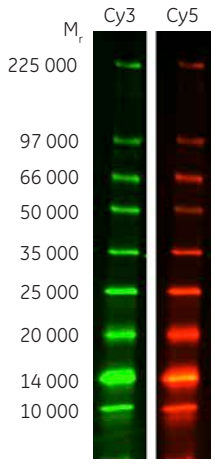


Fig 7. The Amersham WB molecular weight (M_r) markers are visible in both Cy3 and Cy5 channels.

Convenient prelabeling protocols

Three protocols are available for prelabeling of samples: the SDS-PAGE prelabeling protocol; Western blotting prelabeling protocol; and the Quick SDS-PAGE prelabeling protocol.

In the SDS-PAGE prelabeling protocol, the sample is first diluted with Amersham WB labeling buffer and heated to minimize labeling variations. Cy5 dye is then added and the reaction is performed at room temperature for 30 min. To stop the reaction and prepare for electrophoresis, one volume of loading buffer is added and the sample is heated for 5 min.

The Western blotting prelabeling protocol is performed in original cell lysis buffer and the the Cy5 dye is diluted 1:10 to avoid saturated signal on the membrane.

The Quick SDS-PAGE prelabeling protocol is speeded up by allowing the reaction to take place at 95°C for only 5 min. It is mainly recommended for qualitative analysis.

Same results regardless of sample buffer

Dilution of sample in Amersham WB labeling buffer 1:10 neutralizes any differences in labeling efficiency due to buffer components (Fig 8).

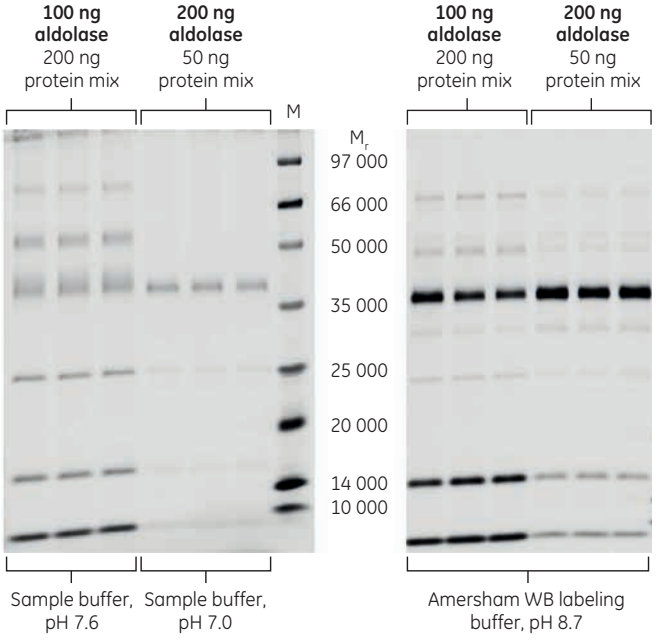


Fig 8. Prelabeling of identical samples in sample buffer pH 7.0 and 7.6 or using the SDS-PAGE labeling protocol with 1:10 dilution in Amersham WB labeling buffer at pH 8.7. M = Amersham WB molecular weight markers.

Buffer exchange prior to labeling

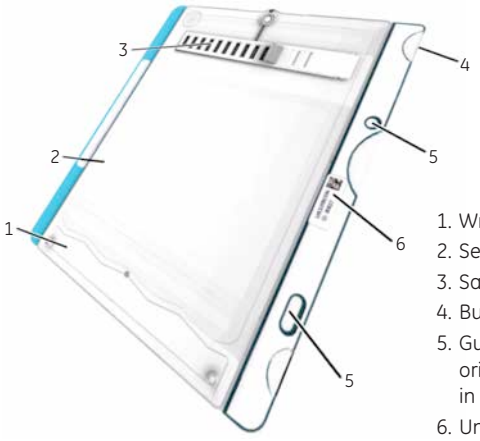
Most buffer components do not interfere with the prelabeling reaction. Those that *do* include imidazole, glycine buffers (> 100 mM), ammonium sulfate, and guanidium chloride. These substances require exchange of buffer, which is easily performed with the Amersham WB MiniTrap™ kit (Fig 9). The kit is designed for rapid and convenient clean-up of protein samples (M_r > 5000) using Sephadex™ G-25 chromatography medium (resin) in disposable columns for separation.

Fig 9. Amersham WB MiniTrap kit.

Amersham WB gel card consumables

Dedicated Amersham WB gel card consumables are available to ensure convenient SDS-PAGE (Fig 10):

- Buffer strips containing running buffer for consistency and convenience.
- Guide holes secure position of the gel card in every step.
- Stacking and separation zone for optimal resolution.
- Horizontal loading of up to 30 µl of sample per well in up to 14 sample wells for convenience.
- Homogenous and gradient gel cards for optimal resolution of proteins ranging between M_r 3500 and 225 000.



1. Writing surface
2. Separation zone
3. Sample wells, 20 µl
4. Buffer strip holder
5. Guiding holes secure orientation and positioning in every step
6. Unique ID for traceability

Fig 10. Amersham WB gel card.

Easy electrophoresis and opening of the gel card

The Amersham WB gel card cassette (Fig 10) and solid buffer format together with horizontal loading of the sample allow fast and easy handling and setup. Electrophoresis is automatically stopped upon detection of the front, followed by automatic scanning of the gel. Evaluation of the results is automatically generated and presented with the possibility for manual editing (Fig 11).

Sample:	Dilution series of LMW containing carbonic anhydrase from 300 ng to 50 pg
Gel card:	Amersham WB gel card 14, 13.5%
Prelabel:	Amersham WB Cy5
Detection:	Cy5 channel
Imaging:	Amersham WB analyzer electrophoresis & scanning unit
Limit of detection (LOD):	50 pg
Dynamic range (DR):	3.8 orders of magnitude

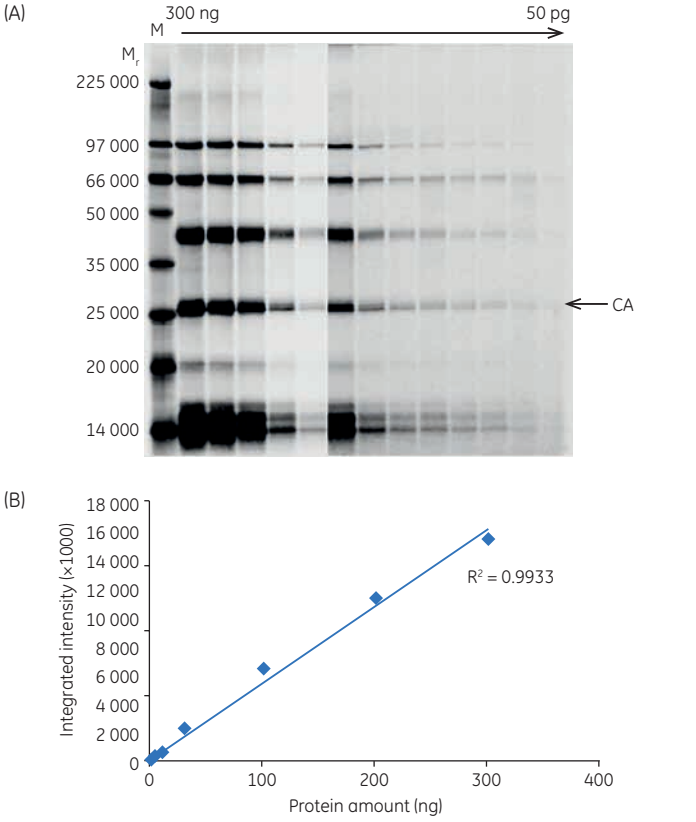


Fig 11. Evaluation of dynamic range and limit of detection for Amersham WB gel card. Detection of Cy5 signals from a prelabeled sample containing carbonic anhydrase (CA) in decreasing amounts. The result demonstrated a dynamic range of 3.8 orders of magnitude and a limit of detection 50 pg. The image is a composite with two different contrast settings for clarity (top). M = Amersham WB molecular weight markers.

When the gel card is run as part of a Western blot workflow it can be opened to expose the separation area to the membrane (Fig 12). The gel is still conveniently attached to a frame allowing easy handling when stacking it with the PVDF membrane card and other components of the transfer sandwich.

The exposed gel separation zone also allows access to allow cutting out bands for mass spectrometry applications.

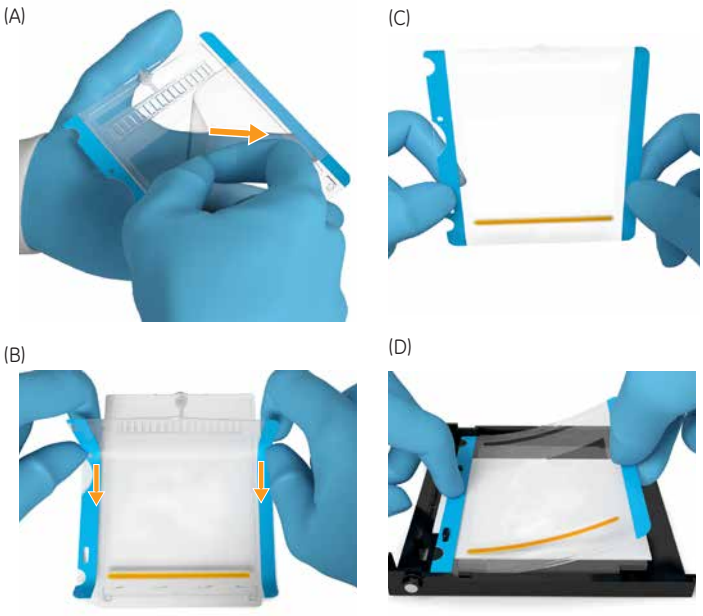
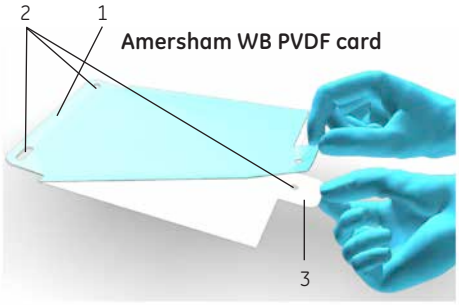


Fig 12. Opening the Amersham WB gel card for Western blotting. (A) The foil lid is removed to expose the separation gel. (B) The gel with frame is separated from the backing. (C) Gel still conveniently attached to frame. (D) Positioning the gel in the transfer sandwich.

Amersham WB Western blot consumables

Consumables for convenient Western blotting including Amersham WB PVDF card and dedicated CyDye labeled secondary antibodies are available (Fig 13):

- Keyed design of the Amersham WB PVDF card and guiding holes for ease-of-use and to eliminate user mistakes.
- Components optimized for low fluorescence and minimal background signal contribution.
- Dedicated secondary antibodies labeled with Cy3 and Cy5.
- Molecular weight markers for detection in both Cy3 and Cy5 channels.



PVDF card, transfer paper, and sponge combine for optimal performance.

Fig 13. Amersham WB Western blot consumables—PVDF card, transfer paper, and sponge—are delivered precut for the transfer holder.

Standardized and optimized Western blotting

All critical processing parameters—protocols, volumes, durations, evaluation settings—have been standardized to give optimal results. Dedicated CyDye labeled secondary antibodies ensure high sensitivity and broad linear dynamic range for accurate, quantitative results (Fig 14).

Sample:	Dilution series of CHO cell lysate
PVDF card:	Amersham WB PVDF card
Blocking:	3% BSA in PBST
Primary antibody:	rabbit anti-MAP Kinase (ERK1/2)
Secondary antibody:	Amersham WB goat anti-rabbit Cy3
Detection:	Cy3 channel
Imaging:	Amersham WB analyzer electrophoresis & scanning unit
Limit of detection (LOD):	40 ng
Dynamic range (DR):	2.6 orders of magnitude

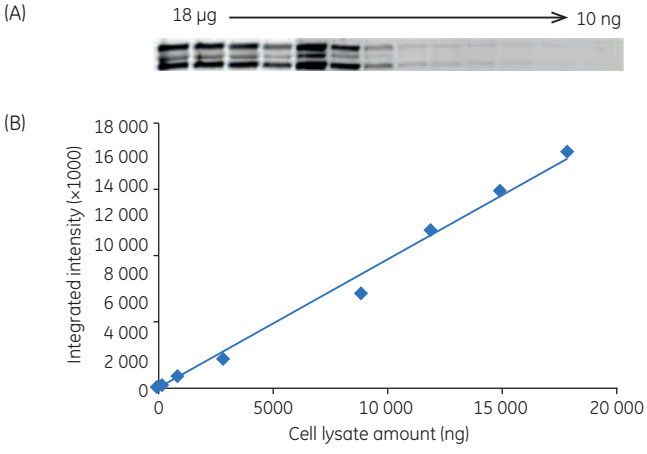


Fig 14. Evaluation of dynamic range and limit of detection for Amersham WB PVDF card. Detection of signals from Amersham WB goat anti rabbit Cy3 antibodies binding to rabbit anti-Erk1/2 in a Chinese hamster ovary (CHO) cell lysate dilution series. The result demonstrated a dynamic range of 2.6 orders of magnitude and a limit of detection 40 ng.

Technical specifications

Amersham WB analyzer

Operating temperature range	15°C to 32°C For full performance: 16°C to 28°C
Relative humidity	20% to 80%, noncondensing For full performance: 20% to 70%, noncondensing
Altitude	Max. 2000 m
Pollution degree	2
Transient level	Overvoltage category II
Environment	Indoor use only
EMC	EN61326-1 and Fcc Part 15B. (Emission according to CISPR 11, Group 1, class B)
System configuration	Benchtop system consisting of external computer and two units. Amersham WB analyzer electrophoresis & scanning unit and Western unit. The computer is not included in the delivery
Operating system	PC with Windows® 7
Control software	Amersham WB software
Sound level	Below 80 dB(A)

Electrophoresis & scanning unit

Dimensions (W × H × D)	47 × 51 × 27 cm
Weight (excluding computer)	25 kg
Power supply	Voltage: 100 to 240 V~ Frequency: 50 to 60 Hz
Power consumption	Max. 300 VA
Fuse	2 × T4AH 250 V

Scanner

Image sensor	Silicon photodiode
Warm-up time	At least 1 min (included in automatic sensitivity mode)
Lens	F1.0/13 mm
Light source	Cy5: Laser diode module, 635 nm, 10 mW Cy3: Laser diode module, 532 nm, 10 mW
Dynamic range	4.6 orders of magnitude
Gray scale	65 536 levels (16 bit)
Image output	Grayscale 16-bit (tif)
Operation	Fully automated (auto exposure, no focus or other adjustment or calibrations needed)

Separation

Voltage	250 to 600 V[1]
Current	20 to 50 mA[1]

[1] Maximum power is 20 W/gel card. The maximum values for the parameters cannot be reached simultaneously.

Western unit

Dimensions (W × H × D)	43 × 39 × 53 cm
Weight	20 kg
Power supply	Voltage: 100 to 240 V~ Frequency: 50 to 60 Hz
Power consumption	Max. power: 400 VA
Fuse	2 × T4AH 250 V
Transfer tubing and connectors	Tubing material: FEP, ID 1/8" Ferrule blue Tubing connector: Nut 5/16"-24UNF 2-A Tubing material: FEP, ID 0.063" Ferrule yellow Tubing connector: Nut 1/4"-28UNF 2-B

Transfer

Voltage	10 to 100 V[1]

[1] Maximum power is 40 W. Maximum current is 400 mA.

Probing

Antibody probing volumes	5 to 12 ml

Drying

Temperature	Max. 45°C
Drying time	10 min

Electrophoresis consumables

Gel card composition	Polyacrylamide/bisacrylamide containing Tris-acetate buffer
Buffer strip composition	Agarose containing Tris-tricine buffer
Amersham WB gel card 13.5%	Separation resolution ($M_r \times 10^3$): 10 to 225
Amersham WB gel card 8–18%	Separation resolution ($M_r \times 10^3$): 6 to 225 (prelabeled proteins) 3.5 to 225 (Western experiments, antibody detection)
Sample loading volume	15 to 30 µl
Sample loading amount	Maximum 20 µg/well < 0.5 µg/protein band
Number of samples	14
Amersham WB molecular weight markers	9 proteins, M_r: 10 000 to 225 000, labeled with both Cy3 and Cy5

Ordering information

Product	Quantity	Code number
Amersham WB analyzer, including electrophoresis & scanning unit, transfer unit, and probing unit	1	29-0320-30
Amersham WB gel card 13.5%	10 gels	29-0225-64
Amersham WB gel card 8–18%	10 gels	29-0225-65
Amersham WB buffer strips	For 10 gels	29-0225-70
Amersham WB PVDF card	For 10 gels	29-0225-66
Amersham WB transfer paper	For 50 blots	29-0639-02
Amersham WB sponge	For 50 blots	29-0341-13
Amersham WB paper comb	For 100 blots	29-0562-86
Amersham WB molecular weight markers	For 10 gels	29-0307-35
Amersham WB labeling buffer	For 10 gels	29-0307-32
Amersham WB loading buffer	For 10 gels	29-0307-33
Amersham WB goat anti-mouse Cy3	For 10 blots	29-0382-75
Amersham WB goat anti-rabbit Cy3	For 10 blots	29-0382-76
Amersham WB goat anti-mouse Cy5	For 10 blots	29-0382-77
Amersham WB goat anti-rabbit Cy5	For 10 blots	29-0382-78
Amersham WB Cy5	For 10 gels	29-0307-31
Amersham WB MiniTrap kit	For 30 blots	29-0222-21
Laptop computer	1	18-1168-52

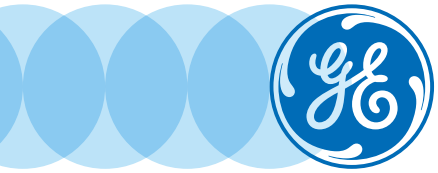
Related literature

	Code number
Application note: Quantitative fluorescence Western blotting using Amersham WB system	29-1138-93
Application note: Analysis of therapeutic antibodies using Amersham WB system	29-1140-27
Handbook: Western blotting, principles and methods	28-9998-97

For local office contact information, visit
www.gelifesciences.com/contact

www.gelifesciences.com

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK



GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

GE Healthcare Europe, GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Japan Corporation
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

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