GE Healthcare
Life Sciences

Amersham* ECL* Gel horizontal electrophoresis system

Amersham ECL Gel and Amersham ECL Gel Box constitute a horizontal mini-gel system for high quality protein electrophoresis. The gel is compatible with standard Laemmli buffers, and has a shelf life of up to 12 months with no compromise in performance over time. Amersham ECL Gel reproducibly resolves complex samples, and works well with standard protein detection protocols. The gel enables high electrotransfer efficiency of proteins, and convenient integration into the Amersham ECL Western blotting workflow.

- Horizontal electrophoresis system with virtually no assembly required
- High lot-to-lot consistency of gels ensures reproducible, high quality results
- Shelf life of up to 12 months allows off-the-shelf selection of the best gel for the application
- Robust gels for convenient handling in downstream detection steps
- Everything required to access and cut the gel is included in the Amersham ECL Gel cassette

The Amersham ECL Gel system (Fig 1) is designed for convenience: the horizontal format enables easy sample loading, low buffer consumption, and no risk of leakage between buffer chambers. In contrast to handcast gels, Amersham ECL Gel is highly reproducible, less time-consuming to prepare and requires no exposure to toxic acrylamide.

Fig 1. Amersham ECL Gel system: Precast gels, gel box, and premixed running buffer for polyacrylamide gel electrophoresis (PAGE) in a convenient, horizontal format.

Amersham ECL Gel is available in 15, 10, and 2 well formats and as either homogeneous (10 and 12%) or gradient (4-12%, 8-16%, and 4-20%) gels. For running SDS-PAGE, a ten-fold concentrated running buffer is available as well as precut Hybond* membranes for Western blotting applications.

The gel contains no sodium dodecyl sulfate (SDS), making it an excellent solution for protein analysis under both native and denaturing conditions, depending on the choice of running buffer.

Easy sample application and gel handling

The Amersham ECL Gel system is designed to make PAGE as easy as agarose gel DNA electrophoresis (Fig 2).

In contrast to vertical electrophoresis systems, the horizontal format provides an "open" gel surface and a bird's eye view of the entire electrophoresis run. Consequently, it is simpler to apply samples to the wells, and samples are easier to visualize once loaded.

For further processing, the gel is simply detached from the gel cassette after electrophoresis. At 1.4 mm, Amersham ECL Gel is somewhat thicker than most other precast and handcast gels, making it easier to handle, with reduced risk of gel breakage, and making downstream processing more convenient. The gel thickness also increases the potential sample loading volume for preparative gel runs, with a maximum volume of 100 µl/well in the two-well gel.

Fig 2A. Amersham ECL Gel comes securely enclosed in a plastic cassette. The entire cassette is placed in the Amersham ECL Gel Box. The comb is removed to expose the wells for sample loading.

Fig 2B. The horizontal format allows ergonomic sample loading and a bird's eye view of the entire electrophoresis run.

Fig 2C. No additional tools are needed; the comb is used as a tool to open the cassette.

Fig 2D. The edge of the upper section of the cassette is used to remove excess gel in preparation for downstream processing.

Gel performance: Extended shelf life

Amersham ECL Gel can be used with confidence for up to 12 months after manufacture. The consistency of results, regardless of storage time is illustrated in Figure 3.

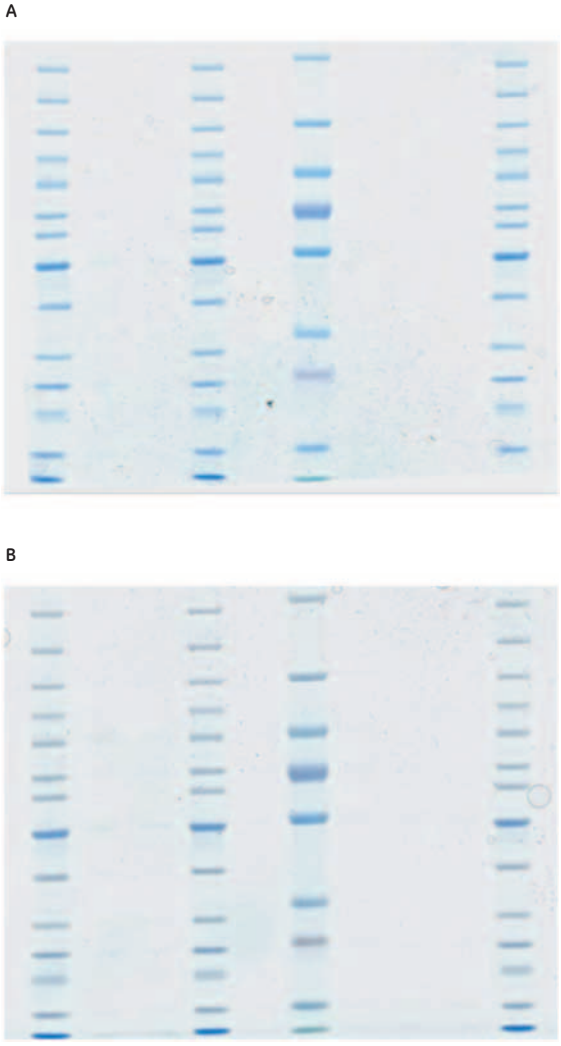

Fig 3. Two 4-12% Amersham ECL Gels from the same batch run on two separate occasions. A = Gel run immediately after manufacture, B = Gel from same the batch run after 12 months storage.

Gel performance: Resolution and sensitivity

Following PAGE, proteins may be visualized by treating the gel with a total protein stain. The most commonly used stain is Coomassie** Blue, but higher sensitivity is achievable with fluorescent stains such as Deep Purple* Total Protein Stain.

In the following example, SDS-PAGE was performed on whole lysates from HeLa cells and purified transferrin. The gels were then stained using Deep Purple and Coomassie Blue (Fig 4). In both cases, the stained gels demonstrate high resolution of the protein samples, making Amersham ECL Gel a suitable method for applications such as Western blotting and purity analysis.

Gel type:	Amersham ECL Gel 12%, 10 wells
Sample:	Two-fold dilution series of HeLa cell lysate (Deep Purple from 500 ng, Coomassie Blue from 1 µg) and transferrin (Deep Purple from 500 ng, Coomassie Blue from 1 µg)
Detection:	(A) Deep Purple, (B) Coomassie Blue
Imaging:	(A) Typhoon* FLA 9000, (B) ImageScanner* III
Analysis:	ImageQuant* TL 7.0

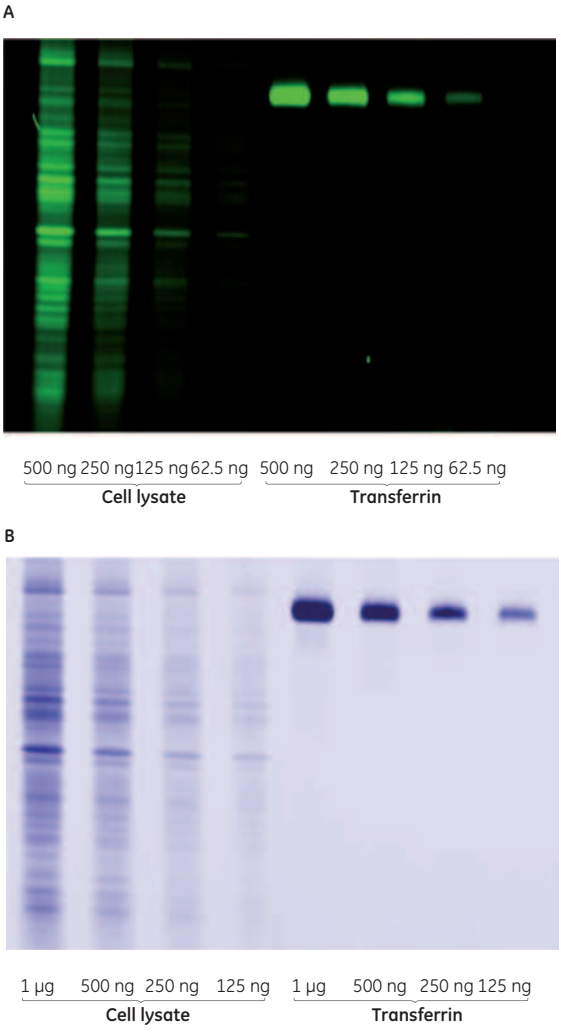

Fig 4. Complex and purified samples after SDS-PAGE on Amersham ECL Gel, stained with either (A) Deep Purple or (B) Coomassie Blue.

Choosing the right gel for the application

Amersham ECL Gel is cast without SDS. This allows the user to define the separation conditions by the composition of the running buffer and sample loading buffer.

The resolution of large proteins by PAGE requires a gel of low polyacrylamide density, while resolution of smaller proteins requires a denser matrix. Resolution of several proteins covering a range of molecular weights is best served using a gradient gel. Amersham ECL Gel is available in a variety of concentrations and the optimal gel should be selected according to the expected sizes of proteins in the sample (Fig 5).

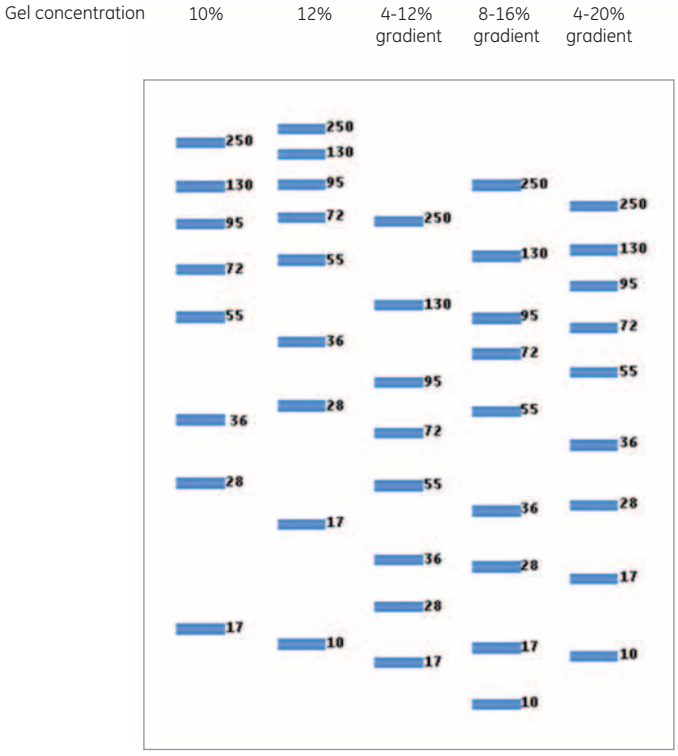

Fig 5. Relative band migration patterns. This diagram shows the relative positions to which proteins of a given molecular weight are expected to migrate in Amersham ECL Gel, depending on the concentration. A typical run time on Amersham ECL Gel is 1 h.

Assessing the purity of IgG from human plasma

Here, SDS-PAGE was performed on samples from different stages of a purification process of human plasma-derived IgG using an ÄKTApilot* system (Fig 6). After PAGE, the gel was stained with Deep Purple Total Protein Stain. The high resolution and sensitivity demonstrates that Amersham ECL Gel is well suited for protein purity analysis following fractionation on ÄKTA systems.

Gel type: Amersham ECL Gel 4-20%, 15 wells
Sample: Plasma fractions from an IgG purification process, 500 ng total protein
Detection: Deep Purple Total Protein Stain
Imaging: Typhoon FLA 9000
Analysis: ImageQuant TL 7.0

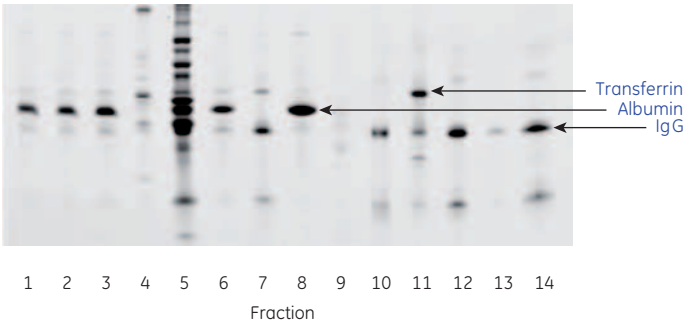

Fig 6. Plasma fractions in an IgG purification process, stained using Deep Purple Total Protein Stain. The enrichment and purification of IgG can be followed through different steps from the original plasma pool (1) to purified IgG (14). Fractions run in lanes 7 and 11 show where albumin and transferrin, respectively, are separated from IgG.

Assessing on-column cleavage efficiency

In this experiment, two different proteases were compared for activity and cleavage efficiency. Glutathione S-transferase conjugated with green fluorescent protein (GST-GFP) was used as test protein. GST-GFP was bound to a GST SpinTrap* column, and after a series of washes, protease A or protease B was added.

The overall yield of eluted pure, non-tagged GFP was approximately 60% for both proteases and the migration pattern was similar on SDS-PAGE using Amersham ECL Gel (Fig 7).

Gel type: Amersham ECL Gel 8-16%, 10 wells
Sample: GST-GFP-His, 1 to 2 µg/well
Detection: Deep Purple Total Protein Stain
Imaging: Typhoon FLA 9000
Analysis: ImageQuant TL 7.0

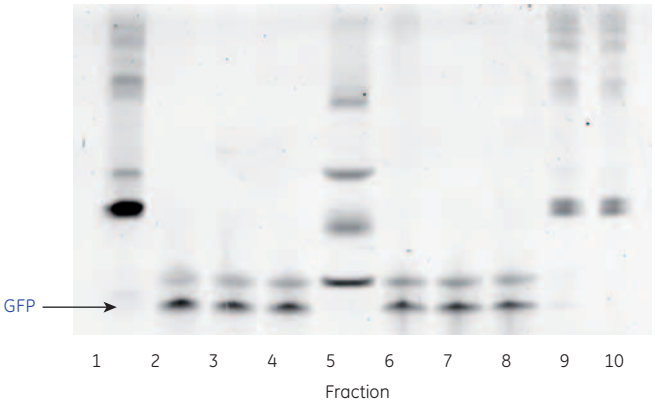

Fig 7. SDS-PAGE of fractions eluted after on-column cleavage of GST-GFP-His. Lane 1: Starting material (GST-GFP-His). Lanes 2-4: Eluates after cleavage with protease A. Lane 5: LMW-SDS Marker Kit. Lanes 6-8: Eluates after cleavage with protease B. Lane 9: Flow-through from GST SpinTrap where protease A was used. Lane 10: Flow-through from GST SpinTrap where protease B was used. The arrow indicates the position of GFP.

Amersham ECL Gel in Western blotting

SDS-PAGE is the most commonly used method for protein separation prior to Western blotting. Electrotransfer from Amersham ECL Gel is highly efficient, with typically in the range of 95% of the protein quantity transferred to the membrane (Fig 8). Amersham ECL Gel provides optimal conditions for high sensitivity Western blotting and is optimized for use with Amersham ECL Prime and ImageQuant LAS 4000 imagers.

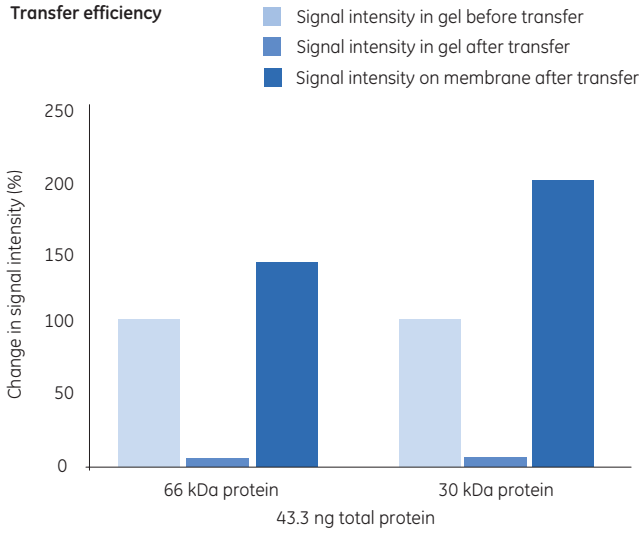

Fig 8. Protein quantity of two proteins included in the LMW-SDS Marker Kit on a PVDF membrane and in Amersham ECL Gel before and after wet transfer. The proteins are transferred efficiently to the membrane and only minute quantities remain in the gel.

Western blotting: Quantitating post-translational modifications

p38 is a mitogen-activated protein kinase involved in cell differentiation and apoptosis, and is regulated by phosphorylation. Here, HEK 293T cells were exposed to transforming growth factor-β (TGF-β). After PAGE of cell lysates on Amersham ECL Gel, Western blotting was performed to evaluate levels of p38 and phosphorylated p38 (pp38) over time (Fig 9). Relative quantitation of pp38 was performed after normalization with levels of the housekeeping protein, GAPDH.

Gel type:	Amersham ECL Gel 4-20%, 10 wells
Sample:	Lysate from HEK 293T cells stimulated with TGF-β for 0, 30 and 120 min. Equal volumes of each sample were loaded in duplicate on one gel
Membrane:	Amersham Hybond-P (PVDF)
Blocker:	5% BSA in PBS-Tween
Primary Abs:	Mouse polyclonal anti-human p38 (1: 5000) Mouse monoclonal anti-human pp38 (1: 5000) Mouse monoclonal anti-GAPDH (1: 2500)
Secondary Ab:	Polyclonal HRP-conjugated anti-mouse IgG (1: 50 000)
Detection:	Amersham ECL Prime
Imaging:	ImageQuant LAS 4000 mini
Analysis:	ImageQuant TL 7.0

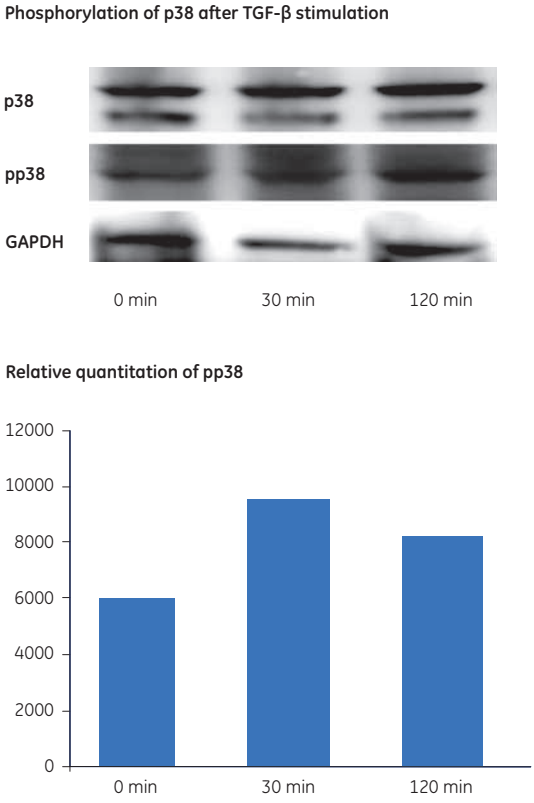

Fig 9. Quantitative Western blotting analysis of p38 and pp38 following stimulation of HEK 293T cells with TGF-β. Data courtesy of Professor Marene Landström, Umeå university, Sweden.

p38 responded to stimulation with TGF-β by phosphorylation after 30 min. Note that pp38 signals in isolation would indicate a peak in phosphorylation levels after 120 min. Normalization by comparison with GAPDH signals shows that this is not the case, but that the presence of pp38 peaks after 30 min. pp38 then remains phosphorylated in the presence of TGF-β.

The results of the Western blotting application presented here show that Amersham ECL Gel provides optimal conditions for protein transfer and analysis, allowing precise quantitation of small changes in protein expression or post-translational modifications.

Ordering information

Product	2 wells (10 gel pack) Code no.	10 wells (10 or 2 gel pack) Code no.	15 wells (10 gel pack) Code no.
Amersham ECL Gel 10%	28-9901-60	28-9898-04 28-9898-08[1]	28-9901-55
Amersham ECL Gel 12%	28-9901-61	28-9898-05 28-9898-09[1]	28-9901-56
Amersham ECL Gel 4-12%	28-9901-62	28-9898-06 28-9901-51[1]	28-9901-57
Amersham ECL Gel 8-16%	28-9901-63	28-9898-07 28-9901-52[1]	28-9901-58
Amersham ECL Gel 4-20%	28-9901-64	28-9901-54 28-9901-53[1]	28-9901-59

[1] 2 gel pack

Product	Code no.
Amersham ECL Gel Box	28-9906-08
Amersham ECL Gel Running buffer, 250 ml	28-9902-52

Related products	Code no.
Hybond-P (8 × 7.5 cm), 10 units	28-9909-83
Hybond-LFP (8 × 7.5 cm), 10 units	28-9909-84
Hybond ECL (8 × 7.5 cm), 10 units	RPN7.58D
Protran* BA83, 0.2 µm Blotting sandwich (8 × 7.5 cm), 10 units	10-4853-85
Protran BA85, 0.45 µm Blotting sandwich (8 × 7.5 cm), 10 units	10-4853-92
Amersham ECL Prime Western Blotting Detection Reagent, for 1000 cm^2 membrane	RPN2232
EPS 301 Power Supply	18-1130-01
Full-Range Rainbow Molecular Weight Markers, 250 µl	RPN800E
Bromophenol Blue, 10 g	17-1329-01
Deep Purple Total Protein Stain, 5 ml	RPN6305

Amersham ECL Gel specifications

Shelf life	12 months from date of manufacture. Refrigerated storage
Gel dimensions	80 × 75 × 1.4 mm
Sample wells	15 (20 µl), 10 (35 µl), or 2 (100 µl) wells
Stacking gel	4%
Gel buffer	Tris-HCl
Running buffer	Native conditions: 25 mM Tris, 192 mM glycine, pH 8.3 Denaturing conditions: 25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3
Sample buffer	Tris-HCl ± SDS or other buffer suitable for the application

Amersham ECL Gel Box specifications

Dimensions	167 × 148 × 43.5 mm (W × H × D)
Maximum voltage	200 V
Maximum power	20 W
Recommended power supply	EPS 301
Operating temperature	4 to 40°C Storage at room temperature
Running buffer consumption	190 ml per gel
Electrophoresis run time	1 h

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imagination at work

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