GE Healthcare

Amersham GST Western Blotting Detection Kit

For the detection of GST fusion proteins on Western Blots using enhanced chemiluminescence

Product Booklet

Code: RPN1237

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1. Legal

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2. Handling

2.1. Safety warnings and precautions

Warning: ***For research use only.*** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

2.2. Storage

The kit is supplied as two boxes. Store anti-GST-HRP and rGST at -15°C to -30°C. Store the box containing ECL™ reagents and ECL blocking agent at 2–8°C. Once open store ECL blocking agent at room temperature.

2.3. Stability

All components are stable for at least 3 months when stored under the recommended conditions.For expiry details see outer packaging information.

3. Components

Anti-GST-HRP conjugate: Horseradish peroxidase (HRP) conjugated to goat anti-GST polyclonal antibody. 75 µl supplied in phosphate buffered saline containing 50% Glycerol. See safety warning section.

rGST positive control: recombinant Glutathione S-transferase. 10 µl supplied at 5 mg/ml protein concentration in Phosphate buffered Saline.

ECL blocking agent: 20 g.

ECL detection reagents: detection reagent 1 (125 ml), detection reagent 2 (125 ml), sufficient for 2000 cm^2 membrane.

4. Other materials required

These materials are not provided with the GST detection kit. All chemicals listed can be obtained from GE Healthcare through the UltraPure and PlusOne™ product ranges.

All buffers should be stable for at least 3 months if prepared in advance and stored at room temperature, however storage at 2–8°C may be necessary to avoid microbial spoilage. Do not use Sodium Azide as a bacteriocide as it inhibits horseradish peroxidase enzymes.

For gel electrophoresis and electroblotting

- Hoefer™ miniVE vertical electrophoresis system or equivalent
- Power pack
- Nitrocellulose membrane, GE Healthcare, Hybond™ ECL RPN2020D
- Appropriate reagents for SDS-PAGE electrophoresis and electroblotting.

For immunodetection

- Phosphate-buffered saline (PBS), pH 7.5

For 1 litre
Dissolve 11.5 g di-Sodium Hydrogen Orthophosphate anhydrous (80 mM), 2.96 g Sodium Dihydrogen Orthophosphate (20 mM) and 5.84 g Sodium Chloride (100 mM) in 1000 ml distilled water. Adjust pH

- Tris-buffered saline
  8 g Sodium Chloride (137 mM) to (TBS), pH 7.6

For 1 litre
Add 20 ml 1M Tris-HCl pH 7.6 (20 mM) and 8 g Sodium Chloride to 1000 ml distilled water. Adjust pH.

- PBS-Tween (PBST) and TBS-Tween (TBST)
  Dilute the required amount of Tween™ 20 in the corresponding buffer. A 0.1% Tween 20 concentration is suitable for most blotting applications.
- Platform shaker
- SaranWrap™ or appropriate plastic wrap.
- Autoradiography film, GE Healthcare, Hyperfilm™ ECL, film cassette and developing system.

5. Description

The Glutathione S-transferase (GST) gene fusion system supplied by GE Healthcare is used for the cloning, expression, purification and detection of fusion proteins.

- pGEX plasmids are designed for inducible, high-level expression of genes or gene fragments as fusions with GST from *Schistosoma japonicum* (1).
- Fusion proteins are easily purified from bacterial lysates by affinity chromatography utilizing GSTs binding affinity for Glutathione.

The GST Western Blotting Detection kit facilitates the detection of GST fusion proteins on Western blots using chemiluminescence. This method may be used for crude bacterial sonicates, column eluates or purified GST fusion proteins. Unlike biochemical assays, this method is not dependent on the functional activity of GST, which can be affected by folding of the fusion protein.

The kit includes an anti-GST antibody conjugated to horseradish peroxidase (HRP) enabling efficient detection of fusion proteins in a single step, a blocking reagent, a positive recombinant GST control and ECL detection reagents. ECL detection utilizes the oxidation of cyclic diacylhydrazides such as luminol to produce light (2,3). The oxidation of luminol is catalysed by HRP in the presence of enhancers such as phenols to increase light output and duration (2). Maximum light emission is obtained at 428 nm, which can be detected by a short exposure to blue-light sensitive autoradiography film.

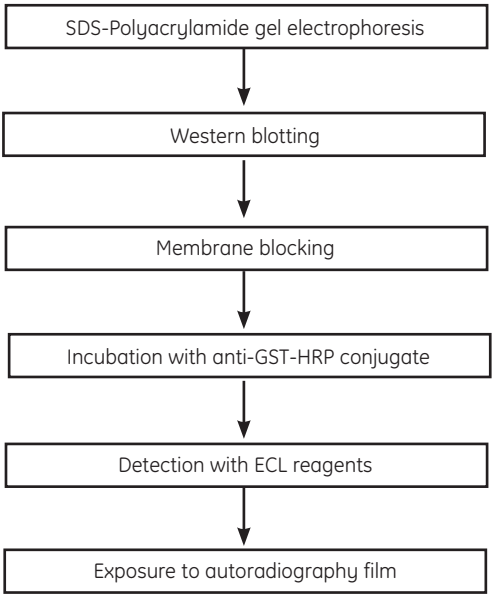

Figure 1. Diagram of work flow

6. Protocol

6.1. Gel electrophoresis

1. Perform SDS-PAGE electrophoresis according to standard techniques (4).

2. Apply a 100 ng loading of the recombinant GST, included in the kit, as a positive control.

3. The anti-GST antibody has been cross-absorbed against *E.coli* proteins. However, this process may not remove all cross-reacting antibodies. We suggest that a sample of an *E.coli* lysate made from a culture that does not contain a pGEX plasmid be run as a control.

6.2. Western blotting

1. Transfer the proteins onto a membrane using standard protocols. Nitrocellulose is recommended, however PVDF has been shown to give comparable results.

2. Hybond ECL should be pre-wetted in distilled water and equilibrated in transfer buffer for 5–10 minutes before blotting. Hybond PVDF should be immersed briefly in 100% Methanol then rinsed in distilled water before equilibration in transfer buffer and blotting.

6.3. Immunodetection

1. Block non-specific binding sites by immersing the membrane in 5% ECL blocking agent in PBST or TBS, (see other materials required), for 1 hour on a platform shaker at room temperature.

2. Briefly rinse the membrane in two changes of PBST or TBST wash buffer.

3. Dilute the anti-GST-HRP conjugate in PBST or TBST. A 1:5000 dilution has been found to be satisfactory for many applications. Allow sufficient antibody solution to cover the membrane (at least 0.25 ml/cm^2 membrane). Incubate the membrane in diluted

conjugate for 1 hour at room temperature on a platform shaker.

4. Briefly rinse the membrane in two changes of PBST or TBST wash buffer. Wash the membrane in >4 ml/cm^2 of wash buffer for 15 minutes at room temperature with gentle shaking.
5. Wash the membrane for 3 x 5 minutes with fresh changes of wash buffer at room temperature with gentle shaking.

6.4. ECL detection

1. Prepare the ECL detection reagents, by mixing an equal volume of solution 1 with solution 2. Allow sufficient volme to cover the membrane (at least 0.125 ml/cm^2 is recommended). Although the mixed reagents are stable for 1 hour at room temperature it is advisable to mix the reagents immediately before use.
2. Drain the excess wash buffer from the washed membrane and place protein side up on a sheet of SaranWrap or other suitable clean surface. Pipette the mixed reagents onto the membrane and incubate for 1 minute.
3. It is necessary to work quickly once the membrane has been exposed to detection reagents. Drain off excess reagents by blotting the edge of the membrane on a tissue. Place the membrane on a fresh piece of SaranWrap, protein side down. Wrap the membrane, taking care to gently smooth out any air bubbles.
4. Place the wrapped membrane protein side up in an X-ray film cassette.
5. Complete further stages in a dark room using red safe lights. Place a sheet of autoradiography film on top of the membrane. Close the cassette and expose for 1 minute.
6. Remove the film and replace with a second sheet of unexposed film. Develop the first piece of film immediately. Dependent on the appearance of the first film estimate the exposure time for the second piece of film, this may vary from 5 minutes to 1 hour.

7. Additional information

7.1. Gel electrophoresis

• Use of purified samples and crude lysates

This conjugate has been used with both purified proteins and crude bacterial lysates. The anti-GST antibody has been cross-absorbed against *E.coli* proteins. However, this process may not remove all cross-reacting antibodies. We suggest that a sample of an *E.coli* lysate made from a culture that does not contain a pGEX plasmid be run as a control.

7.2. Western blotting

• Use of alternative membranes

Nitrocellulose membrane is recommended with this protocol however, PVDF membrane has been shown to give comparable results.

7.3. Immunodetection

• Use of more concentrated anti-GST-HRP conjugate

This protocol has been optimized to give good signal:noise, resulting in intense signal and clean backgrounds. Customers may find sensitivity is improved by increasing the concentration of conjugate used however, this may result in increased background noise.

• Use of ECL Plus detection reagents

ECL detection regents are supplied with this kit however ECL Plus detection reagents have also been used. When using ECL Plus reagents, to reduce background noise to an acceptable level increase conjugate dilution two to four fold.

8. Troubleshooting guide

Problem: No signal

Possible cause	Remedy
1. No transfer of proteins during Western blotting	**1.** Stain gel and membrane with total protein stain to check transfer efficiency. Optimize gel acrylamide concentration, time for transfer and current. Ensure gel and membrane make proper contact during blotting and are orientated correctly with respect to the anode. Check that excess temperatures are not reached during electroblotting, producing bubbles or membrane distortion.
2. No retention of proteins on membrane.	**2.** Assess transfer of proteins (as above). Use a fresh supply of membrane.
3. Problems with detection reagents.	**3.** Ensure reagents are being used correctly. Prepare reagents freshly each time. Store reagents at correct temperature.

Problem: Weak signal

Possible cause	Remedy
1. Protein transfer efficiency is poor.	**1.** Check transfer efficiency as in 1.1.
2. Insufficient protein loaded.	**2.** Load more protein on gel.
3. Exposure time is too short.	**3.** Increase film exposure time, up to 1 hour may be required.
4. Conjugate concentration is too low.	**4.** A 1:5000 dilution is recommended but a more concentrated solution may be required for some applications, try 1:1000.

Problem: Excessive diffuse signal

Possible cause	Remedy
1. Too much protein loaded.	**1.** Reduce the protein loaded.
2. Conjugate concentration is too high.	**2.** A 1:5000 dilution is recommended but a more dilute solution may be required for some applications, try 1:10 000.

Problem: High backgrounds

Possible cause	Remedy
1. Inadequate washing	**1.** Ensure post conjugate washes are performed for a sufficient amount of time with an adequate volume of wash buffer (>4 ml/cm2 membrane).

Problem: High backgrounds *Continued.*

Possible cause	Remedy
2. Inadequate blocking	**2.** Check the blocking buffer has been made correctly. Use freshly prepared blocking buffer each time. Increase the concentration of blocking reagent, try 10%. Use alternative blocking agent, e.g. 1–10% BSA, 0.5–3% Gelatin increase incubation time with blocking buffer.
3. Contaminated blotting equipment or buffers	**3.** Clean equipment. Prepare fresh buffers.
4. Conjugate concentration too high.	**4.** A 1:5000 dilution is recommended but further dilution may be required for some applications.

Problem: Multiple bands are seen

Possible cause	Remedy
1. Conjugate is binding non-specifically to other proteins.	**1.** Include a negative control of expression host not containing expression vector to determine non-specific binding.

9. References

1. Smith, D.B and Johnson, K.S., *Gene* **67**, p.31, (1988).

2. Whitehead, T.P. *et al., Clin. Chem.* **25**, 1531-1546, (1979).

3. Isacsson, U and Watermark, G., *Anal. Chim. Acta.* **68**, 339-362, (1974).

4. Laemmli, U.K., *Nature* **227**, 680-685, (1970).

10. Related products

Vectors	
pGEX expression vectors	27-4805-01
Purification modules	
Bulk GST purification module	27-4570-01
RediPack GST purification module	27-4570-02
GST MicroSpin™ purification module	27-4570-03
GST fusion protein cleavage enzymes	
Factor Xa	27-0849-01
PreScission™ Protease	27-0843-01
Thrombin	27-0846-01
Hoefer miniVE vertical electrophoresis system	80-6418-58
Molecular weight markers	
Full-range Rainbow™ molecular weight markers	RPN800
High-range Rainbow molecular weight markers	RPN756
Low-range Rainbow molecular weight markers	RPN755
Blotting membranes	
Hybond-P PVDF membrane	
Pack of 10 membranes 20 x 20 cm	RPN2020F
Roll of membrane 30 x 3 m	RPN303F
Hybond ECL nitrocellulose membrane	
Pack of 10 membranes 20 x 20 cm	RPN2020D
Roll of membrane 30 x 3 m	RPN303D
ECL Blocking agent	RPN2125
Anti-GST antibody unlabelled	27-4577-01
Anti-GST HRP-labelled antibody	RPN1236
Autoradiography film	
Hyperfilm ECL 18 x 24 cm	RPN3103K
Hyperfilm ECL 8 x 10 inches	RPN2114K

Hyperfilm Autoradiography cassettes	
Hypercassette™ 18 x 24 cm	RPN11642
Hypercassette 8 x 10 inches	RPN11649
Hyperprocesssor™ automatic film processor	RPN1700
ECL mini camera	RPN2069

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RPN1237PL Rev D 2006