



For research use only

ISO9001

## EPD™ Western Reagent

Product Name	Qty	Cat. No.
EPD Western Reagent	1 Kit (200 ml)	EBP-1071

### Description

EPD™ (Enhanced Peroxidase Detection) western Reagent Kit consists of enhanced chemiluminescent substrate for horseradish peroxidase (HRP) on immunoblots.

### Kit contents

1. EPD™ Substrate Solution A 100ml (200ml) and Solution B 100ml (200ml).
2. Rapid protocol

- The kit contains sufficient amount of reagents for 100ml (EBP-1071) or 200ml (EBP-1072) preparations (5x6cm<sup>2</sup>).
- The entire reagents can be stored at 2-8°C.
- This product is guaranteed for 6 months from the date of purchase when handled and stored properly.
- You are reminded that certain components in the solutions may cause bleaching on contact with skin.

### Storage Condition

Store at 2-8°C, and then stable for at least 6 months.

### Additional reagents required

Phosphate-buffered saline (PBS) pH7.5 ; 80 mM Na<sub>2</sub>HPO<sub>4</sub>, 20 mM NaH<sub>2</sub>PO<sub>4</sub>, 100 mM NaCl

Tris-buffered saline (TBS) pH7.5 ; 20 mM Tris-HCl, 137 mM NaCl

Tween-20

PBS-T or TBS-T ; 0.05% to 1% of Tween-20 in PBS or TBS

Blocking reagents (BSA or non-fat dried milk) ; usually 1 - 5% in PBS-T or TBS-T

Primary antibody and HRP-secondary antibody ; It is recommended that the antibody dilution should be optimized empirically to maximize signal to noise

### Critical parameters

1. Proper dilution of primary and secondary antibodies for the best results.
2. Proper blocking and washing to minimize nonspecific signals.

## Quick Blotting Protocol (for mini-blot membrane)

1. Perform electrophoresis and transfer the proteins to nitrocellulose or PVDF membrane.
2. Block membrane for 1 hour with 10 ml of 1%–5% blocking reagent in TBS–T or PBS–T.
3. Incubate the blot for 1 hour in 10 ml of primary antibody diluted as recommended by supplier.
4. Wash the blot 4 x 10 minutes with 20 ml of TBS–T or PBS–T.
5. Incubate the blot for 1 hour in 10 ml of HRP–conjugated secondary antibody diluted as recommended by supplier.
6. Wash the blot 4 x 10 minutes with 20 ml of TBS–T or PBS–T.

## Quick Detection Protocol

1. Mix the **Solution A** and **B** at a 1:1 ratio to make EPD™ western blot detection substrate working solution. (2 ml of working solution is sufficient for mini-blot membrane (5x6 cm<sup>2</sup>))
2. Incubate for 1 min with mild agitation.
3. Wet the blotting membrane (hybridized with HRP–conjugated antibody) for 1 min in substrate working solution.
4. Drain excess reagent and cover the blot with clear plastic wrap.
5. Expose the blot to X–ray film for 10–60 seconds in dark room.

## Possible Troubleshooting

### No signal :

1. No transfer of protein : check protein transfer efficiency by staining gels or membranes.
2. Extremely diluted antibodies : check antibody dilution factor.
3. Expired detection system : check substrate activity (**see Note**).

**Note** : checking detection reagents are working – mix solution A and B (5 µl each) on a parafilm and add 1 µl of serially diluted HRP–conjugated antibody. Visible blue light should be produced in the dark.

### Weak signal :

1. Insufficient protein loaded : check protein concentration loaded .
2. Low affinity antibodies : increase antibody reaction times, decrease concentration of Tween–20.
3. Increase incubation time in detection substrate and exposure time on x–ray film.

### High background :

1. Too high antibody concentration : Further dilute antibodies.
2. Inadequate blocking : check components of blocking solution.  
increase concentration of blocking agent (to 10%).  
increase blocking time.  
increase Tween concentration.
3. Inadequate washing : increase washing times and volume of washing buffers.  
increase Tween concentration in washing buffer.
4. Over exposure : expose the film for a minimum period (see Note).