For research use only

ISO9001

PicoEPD™ Western Reagent

Product Name	Qty	Cat. No.
PicoEPD Western Reagent	1 Kit (200 ml)	EBP-1073

Description

PicoEPD™ (Enhanced Peroxidase Detection) Western Reagent Kit consists of a highly sensitive and stable chemiluminescent substrate for horseradish peroxidase (HRP) on immunoblots. The extremely intense signal output and stable emission of blue light for relatively long time (<12 hr) of PicoEPD™ substrate enables the detection of picogram quantities of antigen in immunoblot and direct or indirect South/Northern blot using HRP-conjugated polynucleotide probe.

Kit contents

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

- The kit contains sufficient amount of reagents for 100ml (EBP-1073) or 200ml (EBP-1074) preparations (5x6㎠).
- The entire reagents can be stored at 2-8°C.
- This product is guaranteed for one year 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 one year.

Additional reagents required

Phosphate-buffered saline (PBS) pH7.5 ; 80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 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.

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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 min 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 min 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 PicoEPD™ western blot detection substrate working solution. (2 ml of working solution is sufficient for mini-blot membrane ($5x6cm^2$))
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 1μl 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.

   Note : Light emission of picoEPD™ detection reagent is gradually increased for first 5 min, peaked for following 2 hours, and gradually decreased to basal level for 12 hours.

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).

   Note : Because of high sensitivity of picoEPD™ detection reagent, blue light may be seen with naked eyes on blotting membranes when normally >10 ng antigens are loaded.
   Snap exposure is necessary.

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