

Peroxidase Stain Kit for Immuno-blotting

Staining Kit for Immuno-blotting

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The quality for certainty.

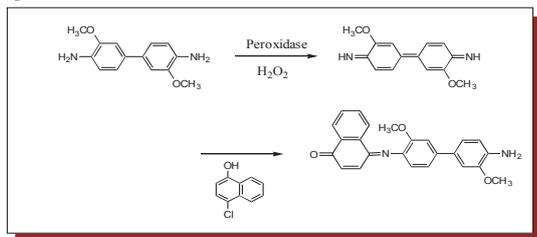


Features

- Highly sensitive:** detect proteins at picogram level
- Clear:** vivid purplish red color
- Low background:** for high contrast

- The product detects peroxidase coupled antibody after blotting (protein or nucleic acid) or in immunohistochemical reaction.
- The bands are stable for more than a month. They do not disappear within several hours or days like as ones stained with 4-chloro-1-naphthol.
- Approx.50-100 pg is the detection limit for Peroxidase. The sensitivity is near to the chemiluminescence method.
- Nuclease tested.

Principle



This detection system utilizes the high sensitivity dyeing reaction of Naphthol and Diamine (Nadi reaction).

Composition

Content	Components	PKG Size	Storage
Staining Stock Solution	Naphthol derivative / Benzidine derivative solution	10ml (1 bottle)	-20°C
Buffer Solution	H3PO4-Citric Buffer Solution, Hydrogen Peroxide	200ml (1 bottle)	4°C

Preparation

Prepare reagents just prior to use, and start reaction within 10 minutes. To leave the prepared reagent for 30 minutes or longer may produce false result. Preparation of staining solution (for membrane size of 10 X 10 cm)

- (1) Shake and mix the buffer solution fully.
- (2) Put 50 ml buffer solution into 50-100 ml measuring cylinder. Add staining stock solution 2.5 ml using micropipette, and mix it fully. For different volume, simply dilute the stock solution by 20 volume of the buffer.

Protocol

- Wash the membrane with the bond peroxidase labeled antibody after Western or dot blotting with Phosphate Buffered Saline (PBS) or Tris Buffered Saline (TBS) containing surfactant.
- Put the prepared staining solution into the clean plastic tray. Immerse the washed membrane into the prepared staining solution, and let it stand or shake it for 10-60 minutes at room temperature.
- When the suitable band appears on the membrane, pull out the membrane from the staining solution, and wash it by running water for more than 10 minutes to stop the reaction.

Application Western blotting :

CBB staining

① ② ③ ④ ⑤

- Albumin
- IgG (heavy chain)
- IgG (light chain)
- Marker

Protocol:

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Separate proteins by electrophoresis
↓
Transfer to membrane
↓
Blocking
↓
Reaction with primary antibody
↓
Wash
                    
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Staining reaction

① ② ③ ④ ⑤

- IgG (heavy chain)
- IgG (light chain)
- Marker

<< Condition >>

Sample	: human serum
Sample amount	: ① 5 µg ② 1.7 µg ③ 0.55 µg ④ 0.2 µg ⑤ 60 ng
Primary antibody	: Anti-human IgG (Goat) POD conjugated
Electrophoresis	: 12.5% SDS-PAGE (35 mA, 40 minutes)
Membrane	: PVDF membrane
Exposure time	: 60 minutes

Reference

1. Tanaka K, Miki Y. 1997. *J Medical Technology* 41: 1020-1024
2. Graham RC JR, Karovsky MJ. 1966. *J Histochem Cytochem* 14: 291-302
3. Chu NM, Janckila AJ, Wallace JH, et al. 1989. *J Histochem Cytochem* 37: 257-263
4. Guthrie JD. 1931. *J Am Chem Soc* 53: 242-244
5. Conyers SM, Kidwell DA. 1991. *Anal Biochem* 192: 207-211

Attention

The use of plastic tray is recommended. Deposits may form in stainless steel tray or container.

Caution

- Carefully wash the measuring cylinder and the plastic tray with water, then in Sulfuric Acid solution of approx. 1 mol/l for more than 30 minutes, and finally with de-ionized water, and let them dry. Contaminated equipment causes false result by reacting with the staining solution.
- The staining solution changes color gradually with time as the result of reduction. Prepare reagents just prior to use, and start reaction within 10 minutes. To leave the prepared reagent for 30 minutes or more may produce false result.
- If the bands spread, wash the membrane with PBS or TBS without surfactant.
- If the concentration of Peroxidase labeled antibody is too high, strong background will appear on the membrane. Use appropriate concentration of the Peroxidase labeled antibody.
- If the rinsing of membrane after staining is not throughout enough, the whole membrane may become dark after drying. Rinse the membrane fully after staining.
- The staining stock solution contains mutagenic substance. If it accidentally comes into contact with skin, thoroughly wash it away with copious amount of water.
- If the bands exposed to strong light, the coloration of bands deepens. Keep the membrane in dark place.
- Discard it according to the regulations in your area.
- Store the product strictly according to the recommended storage condition.

Expiration

One year from manufacturing. Expiration date is stated on the product label (Exp. yy / mm)

Ordering Information

Product name	Grade	Storage	Code No.	PKG Size
Peroxidase Stain Kit for Immuno-blotting	SP	F	26652-70	1 Kit

For research use only, not intended for diagnostic or drug use.

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