GBI Labs
GOLDEN BRIDGE INTERNATIONAL

コスモ・バイオ株式会社

Polink-1 HRP Detection System for Broad Spectrum (for DAB)

(Polymer-HRP detection system, biotin-free, Anti-mouse/rabbit multivalent)
Ready-to-use One Step Polymer Detection System

Storage: 4-8ºC

Catalog No.
- ☐ D11-110 110 ml (bulk, w/o chromogen)
- ☐ D11-60 60ml (bulk, w/o chromogen)
- ☐ D11-18 18 ml (with DAB, good for 150 slides)
- ☐ D11-6 6 ml (with DAB, good for 50 slides)

Intended Use:

Polink-1HRP Broad Spectrum DAB Detection Kit is designed to use with user supplied mouse and /or rabbit antibody to detect target antigen on human tissue or cell samples. Specimen can be frozen or paraffin–embedded tissues, and freshly prepared monolayer cell smears.

Polink-1 1HRP Broad Spectrum DAB Detection Kit is the ONE step polymer detection system that uses polymeric horseradish peroxidase (HRP) -linked goat anti mouse and rabbit IgG to directly detect primary antibody that bound to the tissue. This technology provides excellent sensitivity and high specificity. It is a biotin-free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotin[1]. It is a ONE step detection system that is much faster assay compared to traditional two step method (Biotinylated 2nd antibody, and then streptavidin-HRP). These advantages provide laboratories the benefit of more accurate and quicker result, less trouble shooting and better cost-saving. For AEC staining please choose Polink-1 HRP Broad for AEC (D14-110, D14-18, and D14-6).

Kit components:

Catalog No.	Product Name	**Reagent 1:** Polymer HRP-linked anti-mouse and rabbit IgG (Ready-to-use)	**Reagent 2:** 2A: DAB Substrate 2B: Chromogen concentrate
D11-110	Polink-1 Bulk kit Broad Spectrum	110ml	Not provided
D11-60	Polink-1 Bulk kit Broad Spectrum	60ml	Not provided
D11-18	Polink-1 DAB kit Broad Spectrum	18ml	30 ml of 2A and 2 ml of 2B
D11-6	Polink-1 DAB kit Broad Spectrum	6ml	12 ml of 2A and 1.5 ml of 2B

Recommended Protocol:

1. Fixation: To ensure the quality of the staining and obtain reproducible performance, user needs to supply appropriately fixed tissue and well prepared slides.
2. Tissue need to be adhered to the slide tightly to avoid tissue falling off.
3. Paraffin embedded section must be deparaffinized with xylene and rehydrated with a graded series of ethanol before staining.
4. Cell smear samples should be made as much monolayer as possible to obtain satisfactory results.
5. Investigator needs to optimize dilution and incubation times for primary antibodies.
6. Three control slides will aid the interpretation of the result: positive tissue control, reagent control (slides treated with Isotype control reagent), and negative control.
7. Proceed IHC staining: DO NOT let specimen or tissue dry from this point on.

Reagent	**Staining Procedure**	**Incubation Time (Min.)**
1. Peroxidase Blocking Reagent Supplied by user	a. Incubate slides in peroxidese blocking reagent (Ready-to-use 3% H_2O_2 solution) for 10 min. b. Rinse the slide using distilled water.	10
2. HIER Pretreatment: Refer to antibody data sheet.	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody suggested by vendor. b. Wash with PBS 3 times for 2 minutes each time.	Refer to vendor's data sheet
3. Pre-Block (Optional) Not provided	a. Add 2 (100 µL) or more drops of 10% Normal Goat Serum to cover the tissue section and Incubate 10 min. b. Drain or blot off solution. DO NOT RINSE.	10

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4. Primary antibody: Supplied by user	***Notes:*** Investigator needs to optimize dilution and incubation times a. Apply 2 (100 µL) or more drops of primary antibody to cover the tissue completely. Incubate in moist chamber for 30-60 min. b. Rinse with PBS containing 0.05% Tween-20 3 times for 2 minutes each time.	30-60
5. **Reagent 1:** HRP Polymer-anti-Mouse and anti Rabbit IgG (Ready-to-use)	a. Apply 2 (100 µL) or more drops of HRP Polymer-anti-Mouse/Rabbit IgG to cover tissue section and Incubate in moist chamber for 15 min. c. Rinse with PBS containing 0.05% Tween-20 3 times for 2 minutes each time.	15
6. **Reagents 2A, 2B:** 2A: DAB Substrate 2B: DAB Chromogen	a. Adding 1 drop or 2 drops (for higher contrast) of DAB chromogen concentrate (Reagent 2B) in 1ml of DAB substrate buffer (Reagent 2A). Mix well. b. Apply 2 drops (100 µL) or enough volume of pre-mixed DAB Chromogen to completely cover tissue. Incubate for 5 min. use the prepared DAB solution within 5 hours c. When appropriate color is developed, rinse under tap water gently for about 1-2 minutes.	5
8. Hematoxylin: Supplied by user.	a. Counterstain with 2 (100 ul) or more drops hematoxylin to cover tissue completely and wait about 20 **seconds**. b. Rinse well with tap water for 1-2 min. c. Put slides in PBS until the color turn blue (about 15-30 seconds.) d. Rinse in distill water, then rinse well with tap water	20-30 **seconds**
9. Mounting medium: Supplied by user	Follow the manufacture data sheet procedure for mounting. Recommended product: 1. GB-Mount: Cat. No. E01-15 (15ml), for alcohol soluble substrates (AEC, AP-Red and AP-blue) 2. O-Mount: Cat. No. E02-15 (15ml), for DAB and BCIP/NBT 3. Simpo-Mount: Cat.No. E03-15 (15ml), or E03-100 (100ml), universal permanent mounting medium. Can be used with or without cover slip	Refer to insert

Protocol Notes:

1. The fixation, tissue slide thickness, and primary antibody dilution and incubation time affect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpreting the result.
2. Tissue staining is dependent upon the proper handling and processing of tissues prior to staining. Improper tissue preparation may lead to false negative results or inconsistent results.
3. Do not mix reagents from different lot.
4. Do not allow the slides to dry at any time during staining.

Related Products:

Product	Catalog No.	Size		Product	Catalog No.	Size
Polink-1 HRP Mouse Bulk kit for DAB	D12-110	110ml		*Polink-1 HRP Rat-NM 18ml, 6ml DAB Kit	D35-18 / D35-6	18ml / 6ml
Polink-1 HRP Mouse 18ml, 6ml DAB Kit	D12-18 / D12-6	18ml / 6ml		**Polink-1 HRP Mouse-NR Bulk kit for DAB	D55-110	110ml
Polink-1 HRP Rabbit Bulk kit for DAB	D13-110	110ml		**Polink-1 HRP Mouse-NR 18ml, 6ml DAB Kit	D55-18 / D55-6	18ml / 6ml
Polink-1 HRP Rabbit 18ml, 6ml DAB Kit	D13-18 / D13-6	18ml / 6ml		DAB Kit (2-components)	C09-12	12ml +240ml
Polink-1 HRP Goat Bulk kit for DAB	D33-110	110ml		O-Mount (Organic)	E02-15	15ml
Polink-1 HRP Goat 18ml, 6ml DAB Kit	D33-18 / D33-6	18ml / 6ml		Simpo-Mount (Aqueous)	E03-100 /E03-15	100ml / 15ml
*Polink-1 HRP Rat-NM Bulk kit for DAB	D35-110	110ml				

*Polink-1 HRP Rat-NM kit does not cross react with mouse primary antibody
**Polink -1 HRP Mouse-NR kit does not cross react with Rat primary antibody

Precautious:
Please wear gloves and take other necessary precautions.

Remarks:
For research use or investigation only. Not for diagnostic or therapeutic use.

References:
1. Bisgaard K, Pluzed KP. *Use of polymer conjugates in immunohitochemistry: A comparative study of a traditional staining method to a staining method utilizing polymer conjugates.* Abstract XXI Intl Cong Intl Acad Pathol and 12th World Cong Acad Environ Pathol. Budapest, Hungry, October 20-25, 1996.

2. Shi ZR. Itzkowitz SH, Kim YS. *A comparison of three immunoperoxidase techniques for antigen detection in colorectal carcimoma tissues.* J Hitochem Cytochem 36:317-322,

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