



# Polink-1 AP Detection System for Goat Primary Antibody

(Polymer-AP detection system, biotin-free, Anti-Goat primary antibody)

Ready-to-use One Step Polymer Detection System

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a	Catalog No.	Ш	D61-110	110 ml (bulk, w/o chromogen)
Storage: 4-8°C			D61-18	18 ml (w/o chromogen)
			D61-6	6 ml (w/o chromogen)

#### Intended Use:

Polink-1 AP Goat Detection Kit is designed to use with user supplied Goat 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 AP Goat Detection Kit is the ONE step polymer detection system that uses polymeric alkaline phosphatase (AP) -linked anti Goat IgG to directly detect primary antibody that bound to the tissue. Polink-1 AP Goat Detection Kit does not cross react with bovine IgG. It is compatible with BSA containing diluent or blocking buffer. 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<sup>1</sup>. It is a ONE step detection system that is much faster assay compared to traditional two-step method (Biotinylated 2<sup>nd</sup> antibody, and then streptavidin-AP). These advantages provide laboratories the benefit of more accurate and quicker result, less trouble shooting and better cost-saving.

# Kit components:

Catalog No.	Product Name	Reagent: Polymer AP-linked anti- Goat IgG (Ready-to-use)		
D61-110	Polink-1 AP-polymer Goat Kit	110ml		
D61-18	Polink-1 AP-polymer Goat 18ml kit	18ml		
D61-6	Polink-1 AP-polymer Goat 6ml kit	6ml		

## **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.
- 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	
HIER Pretreatment:	a. Heat Induced Epitope Retrieval (HIER) may be required for primary	Refer to vendor's	
Refer to antibody data sheet.	antibody suggested by vendor. b. Wash with PBS 3 times for 2 minutes each time.	data sheet	
Blocking (Optional)     Not provided	a. Add 2 (100 µL) or more drops of 1% BSA solution to cover the tissue section and Incubate 10 min.	10 min.	
	b. Drain or blot off solution. DO NOT RINSE.		
3. Primary antibody:	<b>Notes:</b> Investigator needs to optimize dilution and incubation times a. Apply 2 (100 μL) or more drops of primary antibody to cover the tissue	30-60 min.	
Supplied by user	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.		

4. Reagent: AP- Polymer anti Goat IgG (Ready-to-use)	<ul> <li>a. Apply 2 (100 μL) or more drops of AP Polymer-anti Goat IgG to cover tissue section and Incubate in moist chamber for 20-30 min.</li> <li>c. Rinse with PBS containing 0.05% Tween-20 3 times for 2 minutes each time.</li> <li>d. Rinse with tap water.</li> </ul>	20-30 min.
5. <b>Chromogen:</b> Supplied by user.	Recommended products: a. Fast-Red kit (Cat. No. C03-60) good for 600 slides b. AP-Red+ kit (Cat. No. C04-8) 40x good for 2000 slides c. BCIP/NBT RTU kit (Cat. No. C05-100, C05-18)	
6. Hematoxylin:	a. Counterstain with 2 (100 ul) or more drops hematoxylin to cover tissue completely and wait about 20 <b>seconds</b> .	20-30 seconds
Supplied by user.	<ul> <li>b. Rinse well with tap water for 1-2 min.</li> <li>c. Put slides in PBS until the color turn blue (about ½ - 1 min.)</li> <li>d. Rinse in distill water, then rinse well with tap water</li> </ul>	
7. Mounting medium:	Follow the manufacture data sheet procedure for mounting. Recommended product:	Refer to insert
Supplied by user	1. GB-Mount: Cat. No. E01-18 (18ml), for alcohol soluble substrates (AEC, AP-Red and AP-blue) 2. O-Mount: Cat. No. E02-18 (18ml), for DAB and BCIP/NBT 3. Simpo-Mount: Cat.No. E03-18 (18ml), or E03-100 (100ml), universal permanent mounting medium. Can be used with or without cover slip	

# **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 AP Mouse Bulk kit	D18-110	110ml	**Polink-1 AP Mouse-NR Bulk kit	D57-110	110ml
Polink-1 AP Mouse 18ml, 6ml Kit	D18-18 / D18-6	18ml / 6ml	**Polink-1 AP Mouse-NR 18ml, 6ml Kit	D57-18 / D57-6	18ml / 6ml
Polink-1 AP Broad Bulk kit	D17-110	110ml	Fast Red Kit	C03-60	12 Tab + 60ml
Polink-1 AP Broad 18ml, 6ml Kit	D17-18 / D17-6	18ml / 6ml	AP-Red+ Kit (40x concentrate)	C04-8	8ml
Polink-1 AP Rabbit Bulk kit	D19-110	110ml	BCIP/NBT Kit	C05-100/C05-18	100ml / 18ml
Polink-1 AP Rabbit 18ml, 6ml Kit	D19-18 / D19-6	18ml / 6ml	GB-Mount (Aqueous)	E01-18	18ml
*Polink-1 AP Rat-NM Bulk kit	D62-110	110ml	Simpo-Mount (Aqueous)	E03-100 /E03-18	100ml / 18ml
*Polink-1 AP Rat-NM 18ml, 6ml Kit	D62-18 / D62-6	18ml / 6ml			

<sup>\*</sup>Polink-1 AP Rat-NM kit does not cross react with mouse.

### **Precautious:**

Please wear gloves and take other necessary precautions.

### Remarks:

For research use only.

### References:

- 1. <u>Bisgaard K, Pluzed KP</u>. Use of polymer conjugates in immunohitochemistry: A comparative study of a traditional staining method to a staining method utilizing polymer conjugates. <u>Abstract</u> XXI Intl Cong Intl Acad Pathol and 12<sup>th</sup> 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,

<sup>\*\*</sup>Polink-1 AP Mouse-NR kit does not cross react with Rat.