



# Polink-2 Plus HRP Anti- Goat DAB Detection Kit

(2-step Polymer-HRP detection system, biotin-free,)

Polymer Detection System with Super Sensitivity and Specificity

Storage: 4-8°C	Catalog No.	☐ D43-110 ☐ D43-18 ☐ D43-6	110 ml (bulk, w/o chromogen) 18 ml (with DAB, good for 180 slides) 6 ml (with DAB, good for 50 slides)

#### Intended Use:

Polink-2 Plus HRP anti Goat DAB Detection Kit is the 3rd generation of polymer detection system. It uses Goat antibody specific enhancer to help amplify the polymer-enzyme conjugate reaction to achieve super sensitivity and specificity in immunohistochemistry staining. It produces consistent immunostaining outcomes on archival tissues and on difficult-to-work antibodies. User may need to further dilute primary antibody due to super sensitivity of Polink-2 Plus detection system. Polink-2 Plus HRP anti Goat DAB Detection Kit does not cross react with bovine IgG. It is compatible with BSA containing diluent or blocking buffer. It is a biotin-free system, therefore, overcomes the non-specific staining caused by streptavidin/biotin system due to endogenous biotin. Most commonly used specimens for this system are: frozen tissue, paraffin-embedded tissue, freshly prepared lymphocytes and fixed culture cells. It can be used for manual stain or autostainer. Staining conditions need to be optimized by user.

Polink-2 Plus HRP for DAB Detection System offers a wide choice for primary antibodies, including broad spectrum (for mouse and rabbit primary antibodies), mouse, rabbit, goat, and rat primary antibodies. Refer to **Related Product** section for details.

#### Kit components:

Catalog No.	Product Name	Reagent 1: Goat Ab. Enhancer (Ready-to-use)	Reagent 2: Polymer HRP for Goat (Ready-to-use)	Reagent3A, 3B: 3A: DAB Substrate (Ready-to-use) 3B: DAB Chromogen Concentrate
D43-110	Polink-2 Plus HRP Goat for DAB Bulk kit	110ml	110ml	Not included
D43-18	Polink-2 Plus HRP Goat DAB 18ml kit	18ml	18ml	30ml of DAB Substrate 3A 2ml of DAB Chromgen 3B
D43-6	Polink-2 Plus HRP Goat DAB 6ml kit	6ml	6ml	12ml of DAB Substrate 3A 1.5ml of DAB Chromgen 3B

#### **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. Staining steps: DO NOT let specimen or tissue dry from this point on.

Reagent	Staining Procedure	Incubation
		Time (Min.)
1. PEROXIDASE BLOCKING	a. Incubate slides in PEROXIDASE BLOCKING REAGENT (Ready-to-use 3% $\rm H_2O_2$	10
REAGENT. Supplied by user	solution) for 10 minutes.	
	b. Rinse the slide using distilled water.	
2. HIER PRETREATMENT:	a. Heat Induced Epitope Retrieval (HIER) may be required for primary antibody	
	suggested by vendor. Please check the data sheet of primary antibody	
	b. Wash with PBS 2 min., 3 times.	
3. PRIMARY ANTIBODY	a. Apply 2 drops (100 µL) or enough volume of PRIMARY ANTIBODY to cover the	30-60
Supplied by user	tissue section completely. Incubate in moist chamber for 30-60 min.	
	b. Rinse with PBS for 2 min., 3 times.	

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4. Goat Antibody Enhancer (Ready-to-	a. Apply 2 drops (100 µL) or enough volume of Goat Antibody Enhancer to cover	10
use).	each section. Incubate in moist chamber for 10 min.	
Reagent 1		
	b. Rinse with PBS for 2 min., 3 times.	
5. POLYMER-HRP for Goat (Ready-to-	a. Apply 2 drops (100 µL) or enough volume of POLYMER-HRP for GOAT to cover	10
use)	each section. Incubate in moist chamber for 10 min.	
Reagent 2		
	b. Rinse with PBS for 2 min, 3 times.	
6. CHROMOGEN	a. Add 1 drop or 2 drops (for higher sensitivity and contrast) of Reagent 3B into 1ml of	5
Reagent 3A: DAB Substrate	reagent 3A. Mix well. Protect from light and use within 5 hours.	
Reagent 3B: DAB Chromogen	b. Apply 2 drops (100 µL) or enough to completely cover tissue, of pre-mixed DAB to	
	each section. Incubate for about 5 min. Monitor the color development under	
	microscope.	
	c. Rinse with tap water for 1-2 min.	
7. HEMATOXYLIN	a. Counterstain with 2 drops (100 ul) or enough volume of Hematoxylin to cover tissue	15-20
Supplied by user	completely and wait about 15-20 seconds.	seconds
	b. Rinse well under tap water for 1-2 minutes.	
	c. Put slides in PBS until show blue color (about 30-60 seconds).	
	d. Rinse well in distill or tap water	
8. Mounting medium:	Follow the manufacture data sheet procedure for mounting.	Refer to insert
Supplied by user	Recommended product:	
	1. GB-Mount: Cat. No. E01-18 (18ml), for alcohol soluble substrates (DAB, 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:**

The fixation, tissue slide thickness, and primary antibody dilution and incubation time effect results significantly. Investigator needs to consider all factors and determine optimal conditions when interpret the result.

### **Related Products:**

Product	Catalog No.	Size	Product Catalog No.	Size
Polink-2 Plus HRP Mouse for DAB bulk kit (without chromogen)	D37-110	110ml	Polink-2 Plus HRP Rat-NM for DAB (No mouse ) Bulk kit (without chromogen)	110ml
Polink-2 Plus HRP Mouse DAB kit	D37-18 D37-6	18ml 6ml	Polink-2 Plus HRP Rat-NM (No mouse) D46-18 DAB kit D46-6	18ml 6ml
Polink-2 Plus HRP Rabbit for DAB Bulk Kit (without chromogen)	D39-110	110ml	Polink-2 Plus HRP Mouse-NR (No Rat) D58-110 Bulk kit (without chromogen)	110ml
Polink-2 Plus HRP Rabbit DAB kit	D39-18 D39-6	18ml 6ml	Polink-2 Plus HRP Mouse-NR (No Rat) D58-18 DAB kit D58-6	18ml 6ml
Polink-2 Plus HRP Broad for DAB Bulk Kit (Without chromogen. For Ms. & Rb.)	D41-110	110ml	DAB Kit (20x concentrated) C01-12	12ml
Polink-2 Plus HRP Broad DAB Kit (for mouse and Goat antibody)	D41-18 D41-6	18ml 6ml	DAB+ kit C09-12	12ml+240ml

**Precautious:** DAB may be carcinogenic. Please wear gloves and take other necessary precautions.

**Remarks:** For research use only.