HRP Polymer anti-Rabbit IgG Kit for IHC Detection (with Chromogen)

Ready-to-use IHC kit that employ HRP polymer technology to provide increased sensitivity and detection of antigens in samples.

Catalog number: ARG80967

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION **Page**

PRINCIPLE OF THE ASSAY 3
MATERIALS PROVIDED & STORAGE INFORMATION 4
MATERIALS REQUIRED BUT NOT PROVIDED 4
REAGENT PREPARATION 4
ASSAY PROCEDURE 5

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

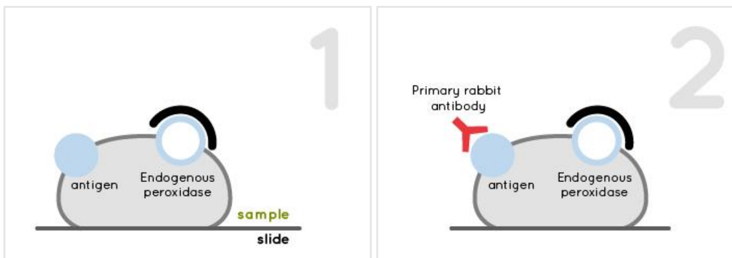
Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

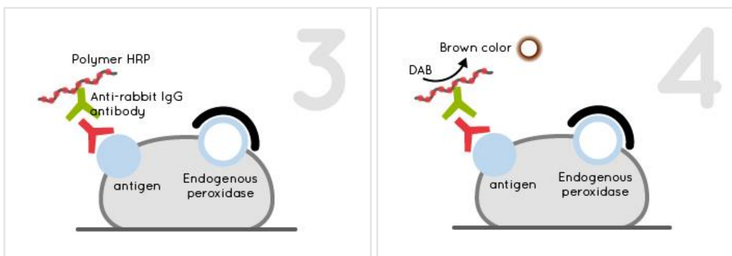
PRINCIPLE OF THE ASSAY

This is a ready-to-use IHC kit that employ HRP polymer technology to provide increased sensitivity and detection of antigens in samples. HRP polymer is conjugated to anti-rabbit IgG heavy and light chain and used as secondary antibodies in Immunohistochemistry or Immunocytochemistry experiments. This detection method greatly reduces high background generated in biotin/avidin systems and gives enhanced signals during color development using DAB substrate reagent. The workflow is simple as illustrated below:



(1) Block endogenous peroxidase and non-specific proteins.

(2) Incubate with primary antibody.



(3) Signal is amplified by rabbit IgG secondary antibody conjugated with HRP polymer.

(4) Color development with DAB.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Bring reagents to RT before use. Store opened reagents at 2-8 °C.

Component	Quantity	Storage information
Peroxidase Blocker (Hydrogen Peroxide)	1 bottle (7 ml) (Ready-to-use)	4°C
Protein Blocker	1 bottle (7 ml) (Ready-to-use)	4°C
Primary antibody dilution buffer	1 bottle (12 ml)	4°C
HRP polymer anti-rabbit IgG	1 bottle (7 ml)	4°C
DAB buffer	1 bottle (7 ml)	4°C
DAB Chromogen Concentrate (20X)	1 bottle (2 ml)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Washing buffer
- Antigen retrievers
- Primary antibody
- Counter stain and mounting medium

REAGENT PREPARATION

- 1X DAB Chromogen Reagent: Add 2 drops (50 µl) of DAB Chromogen Concentrate into 1 ml of DAB buffer in a tube. Mix well. The diluted DAB Chromogen reagent is stable for 7-8 hours.

ASSAY PROCEDURE

Bring reagents to RT before use. Positive and negative controls should be included simultaneously.

For Frozen, Paraffin sections and Cell Smears:

1. Deparafinize and hydrate tissue sections through xylene or other clearing agents and graded alcohols. (Fixation and permealization method should be optimized for each primary antibody being used).
2. Rinse 2-3 times with distilled water.
3. Incubate paraffin sections with Peroxidase Blocker (1-3 drops to cover section) for 10 minutes at RT. For frozen sections, use Peroxidase Blocker (prediluted 1:10 in methanol).
4. Rinse slides 3 times with distilled water.
5. <u>If antigen retriever is required, it can be applied at this step. Please refer to datasheet of primary antibody.</u>
6. Wash slides 3 times with PBS or Tris saline containing 0.02-0.05% nonionic detergent such as Tween-20, NP-40 or Triton X-100.
7. Incubate slide in Protein Blocker for 10 minutes at RT. Do not rinse the slide.
8. Remove Protein Blocker and incubate slides with primary antibody diluted with Primary antibody dilution buffer in a ratio recommended by supplier. The primary antibody dilution buffer can also be used as negative control.
9. Wash slides 5 times with PBS.
10. Incubate with HRP polymer anti-rabbit IgG for 15 minutes at RT.
11. Wash slides 5 times with PBS.

12. Wash slides with distilled water 2-3 times.
13. Incubate with DAB chromogen reagent for 5-10 minutes at RT. Monitor color change under microscope periodically.
14. Wash slides 5 times with distilled water.
15. Incubate with appropriate counterstain (not provided).
16. Wash slides 5 times with distilled water.
17. Mount slides with mounting medium.

** Peroxidase activity can be destroyed by Sodium Azide. Avoid Sodium Azide in all buffers and reagents used.

** The protocol outlines guidelines of procedures. Optimization process should be carried out for each primary antibody and sample type used.