Pat Lab[®]

ANTI-POLYVALENT HRP/AEC SYSTEM KIT

INTENDED USE:

For In Vitro Diagnostic Use

| CATALOG NO | VOLUME |
|------------|-------------------|
| PL-015-HA | 15 ml(150 slides) |

SPECIFICITY : Anti-Mouse IgG , Anti-Rabbit IgG

ENZYME : Peroxidase

CHROMOGEN/SUBSTRATE: 3-amino-9-ethylcarbazole (AEC)

REAGENTS:

| Quantity | Component | Volume | PL-015-HA |
|----------|------------------------------|--------|------------|
| 1 | Protein Block | 15 ml | PL-015-PBQ |
| 1 | Biotinylated Anti-Polyvalent | 15 ml | PL-015-BN |
| 1 | Streptavidin Peroxidase | 15 ml | PL-015-HR |
| 1 | Hydrogen Peroxide Block | 15 ml | PL-015-HP |
| 1 | AEC Chromogen | 2 ml | PL-002-HAC |
| 1 | AEC Substrate | 15 ml | PL-015-HAS |

DESCRIPTION

The reagents in this kit constitute a labeled streptavidin-biotin immunoenzymatic antigen detection system. This technique involves the sequential incubation of the specimen with an unconjugated primary antibody specific to the target antigen, a biotinylated secondary antibody that reacts with the primary antibody, enzyme-labeled streptavidin, and substrate chromogen.

PRINCIPLE OF THE PROCEDURE

This HRP detection system detects a specific antibody bound to an antigen in tissue sections. The specific antibody is located by a biotin-conjugated secondary antibody. This step is followed by the addition of a streptavidin-enzyme conjugate that binds to the biotin present on the secondary antibody. The specific antibody, secondary antibody, and streptavidin-enzyme complex is then visualized with an appropriate substrate/chromogen.

WARNINGS & PRECAUTIONS

Refer to MSDS.

STORAGE & SHELF LIFE

Store at 2-8°C. Product is stable for 24 months from the date of manufacture.

MICROBIOLOGICAL STATE

Product(s) not sterile.

MATERIALS REQUIRED BUT NOT PROVIDED

Primary antibody.

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SPECIMEN & REAGENT PREPARATION

Refer to Procedure.

PROCEDURE

STAINING PROTOCOL (kit components in bold):

1. Deparaffinize and rehydrate tissue section.

2. To reduce nonspecific background staining due to endogenous peroxidase, incubate slide in **Hydrogen Peroxide Block** for 10-15 minutes.

3. Wash 2 times in buffer.

4. If required, incubate tissue in digestive enzyme (or appropriate pretreatment).

5. Wash 4 times in buffer.

6. Apply Protein Block and incubate for 5 minutes at room temperature to block nonspecific background staining.

NOTE: Do not exceed 10 minutes or there may be a reduction in desired stain.

7. Rinse (Optional).

8. Apply primary antibody and incubate according to manufacturer's protocol.

9. Wash 4 times in buffer.

10. Apply Biotinylated Goat Anti-Polyvalent and incubate for 10 minutes at room temperature.

11. Wash 4 times in buffer.

12. Apply Streptavidin Peroxidase and incubate for 10 minutes at room temperature.

13. Rinse 4 times in buffer.

14. Add 1 drop of **AEC Chromogen** to 1 ml of **AEC Substrate** and mix well. Apply mixture to tissue section within 20 minutes of mixing. Incubate tissue section for 5-20 minutes, depending on the desired stain intensity.

WARNING: AEC is a suspected carcinogen. Handle with care and dispose of according to all regulations. 15. Counterstain and coverslip.

The specificity and sensitivity of antigen detection is dependent on the specific primary antibody used.

Troubleshooting

Please contact PatoLab Technical Support by e-mail (patolab@patolab.com.tr).

