Anti-PE, HRP-Labeled Antibody

Produced in Goat

Catalog No.	Size
04-40-02	0.1 mg

DESCRIPTION

Affinity purified goat-derived antibody to Phycoerythrin (PE) was labeled with peroxidase by the periodate method of Nakane and Kawaoi (1). It is designed for the detection of PE in immunohistochemistry (IHC) applications.

FORM/STORAGE

Lyophilized. Store at 2-8 °C. Stable for a minimum of 1 year from date of receipt when stored at 2-8 °C.

STABILIZER AND PRESERVATIVE

Goat serum added as a protein stabilizer. No preservative added. Additional biological protection may be provided with 0.01% thimerosal. DO NOT USE SODIUM AZIDE. Non-sterile.

ANTIBODY CONCENTRATION

The amount of affinity purified antibody is 0.1 mg as determined by UV absorbance at 280 nm.

E/P RATIO

Molar enzyme/antibody protein ratio = 4:1

REHYDRATION AND STORAGE

Note: Rehydration of antibodies in TBS or buffers other than those listed here is not recommended.

Procedure A: 50% Glycerol

At a working dilution, the concentration of glycerol is too small to affect most assays. The use of glycerol is not recommended when the antibody is used in live cell work.
Rehydration: Add 1 mL of 50% glycerol in water to the vial. Pipette up and down several times to ensure proper mixing.
Storage: This product may be stored either refrigerated or at 20 °C. Stable for a minimum of 1 year.

Procedure B: KPL's HRP Stabilizer

Rehydration: Rehydrate with 1 mL of KPL's HRP Stabilizer (see RELATED PRODUCTS). Rotate the vial until the lyophilized pellet is totally dissolved.
Storage: This product should be stored at 2-8 °C. Stable for a minimum of 1 year.

Procedure C: Reagent Quality Water

Rehydration: Add 1 mL of reagent quality water to the vial. Pipette up and down several times to ensure proper mixing.
Storage: This product may be stored aliquotted at -20 °C. Care should be taken to minimize multiple freeze/thaw cycles.

NOTES

1. This antibody is intended to be used for the detection of PE-labeled antibodies in IHC assays.
2. Minimize procedures that may destroy epitopes.
   a. Universal Block is not recommended for inactivation of endogenous enzyme activity, as it may destroy epitopes.
3. Secondary antibodies should be titrated to determine proper working dilutions.
4. Always incorporate a positive control, negative control and reagent control.
5. Do not allow sections to dry out during incubations.
6. Remove as much buffer as possible after washes.
7. Reagent quality water is recommended for use.

PROCEDURES

The protocol below is designed as a starting point for use on frozen sections with colorimetric detection. Assay optimization will need to occur when using this antibody on other specimen types.

FIXATION

1. Fix the section (ex. – acetone, methanol), and block for endogenous enzyme activity, using a block that will not destroy epitopes (ex. – 1% H_2O_2).

APPLY SERUM BLOCK

1. Shake off buffer and wipe off excess buffer surrounding the section.
2. Completely cover section with serum that matches the species the primary antibody was raised in (ex. rabbit or mouse).
3. Incubate 15 minutes at room temperature in a humidified chamber.

APPLY PE-LABELED PRIMARY ANTIBODY

1. Shake off serum block and wipe off any excess surrounding the section.
2. Dilute primary antibody to requisite concentration in TBS containing 1% Normal Serum (use same

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serum species used in previous steps). If performing a dual stain, dilute other tagged primaries during this step as well.
3. Completely cover section with diluted primary antibody (or antibodies).
4. Incubate 10 - 30 minutes at room temperature in a humidified chamber.
5. Rinse off primary antibody with TBS.
6. Soak 5 minutes in same buffer.

APPLY ANTI-PE, HRP-LABELED ANTIBODY

1. Shake off TBS and wipe off any excess surrounding the section.
2. Completely cover section with Anti-PE, HRP-labeled antibody (diluted 1:100 – 1:1,000) in TBS containing 1% Normal Goat Serum. If performing a dual stain, dilute other conjugates during this step as well.
3. Incubate 10 - 30 minutes at room temperature in a humidified chamber.
4. Rinse off antibody with TBS.
5. Soak 5 minutes in same buffer.

COLOR DEVELOPMENT

Develop color using one or more of KPL's HistoMark® substrates (See RELATED PRODUCTS).

NOTE: If color develops too rapidly for your staining conditions, (i.e. less than one minute), further dilution of the primary antibody is recommended. An estimation of appropriate primary antibody dilution may be obtained by applying 1/50, 1/100, 1/200, 1/400 and 1/800 dilutions to tissue sections. The optimal dilution is the one that results in appropriate color development within 10 minutes without background staining.

REFERENCES

1. Nakane, P K., and Kawaoi, A. (1974). J. Histochem. Cytochem. 22:1084.

RELATED PRODUCTS

Anti-PE Antibody	Cat. No. 01-40-02
ReserveAP, anti-PE	Cat. No. 051-40-02
Anti-FITC	Cat. No. 01-40-01
HRP, anti-FITC	Cat. No. 04-40-01
ReserveAP, anti-FITC	Cat. No. 051-40-01
Anti-APC	Cat. No. 01-40-03
HRP, anti-APC	Cat. No. 04-40-03
ReserveAP, anti-APC	Cat. No. 051-40-03
Anti-PerCP	Cat. No. 01-40-04
HRP, anti-PerCP	Cat. No. 04-40-04
ReserveAP, anti-PerCP	Cat. No. 051-40-04
HistoMark TrueBlue	Cat. No. 50-78-02
HistoMark Black	Cat. No. 54-75-00
Normal Goat Serum	Cat. No. 71-00-27
Normal Rabbit Serum	Cat. No. 71-00-28
Normal Mouse Serum	Cat. No. 71-18-01
10% BSA	Cat. No. 50-61-00
10X TBS	Cat. No. 51-17-01
HRP Stabilizer	Cat. No. 54-15-00

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