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Product datasheet for AP31660SU-N

PMN Rabbit Polyclonal Antibody

Product data:

Product Type:	Primary Antibodies
Applications:	CT, IHC
Recommended Dilution:	Immunohistochemistry on frozen and paraffin sections. Cytotoxic assays. For Cytotoxic Antibodies: Modified Colormetric Microtiter Assay 1 Results: Antisera of this antibody diluted 1/50 exhibits 80% cytotoxicity on mouse PMN. Antisera of this antibody diluted 1/50 exhibits <5% cytotoxicity on mouse thymocytes or splenocytes. For Agglutinating Antibodies: Antisera dilutions in RPMI-1640 incubated with target cells at 4°C-8°C for 1hr. Agglutination determined by microscopic observations. Results: Antisera of this antibody strongly agglutinates mouse PMN but not thymocytes at dilutions to 1/100.
Reactivity:	Mouse
Host:	Rabbit
Clonality:	Polyclonal
Specificity:	This antibody reacts to PMN.
Formulation:	State: Serum State: Liquid serum
Conjugation:	Unconjugated
Storage:	Store the antibody at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Synonyms:	Polymorphonuclear Leukocytes
Note:	Protocol: **Rabbit anti-Mouse PMN IHC protocol** **Sections:** Animals were perfused with 10 mM PBS, pH 7.4 followed by 4% paraformaldehyde in 100 mM phosphate buffer, pH 7.4. Tissue was removed and placed in 4% paraformaldehyde for 6 hours at room temperature, then transferred to 10 mM PBS, pH 7.4 in 0.15M isotonic saline

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at 4°C overnight. Sections were cut at 50 µm on a vibratome using 10 mM PBS and placed in tissue culture wells at 4°C overnight.

Procedure:

1. Pretreat slices in 50 mM NH4Cl (0.267g/100 ml PBS) in PBS at 22°C for 1 hour.
2. Pretreat slices in 0.1% Triton X-100 in PBS at 22°C for 1 hour.
3. Wash in PBS for 5 minutes at 22°C.
4. Block in PBS with 5% NGS at 22°C for 2 hours.
5. Incubate in primary antibody (Rabbit anti-mouse PMN), AP31660SU-N, diluted 1/3000 in PBS with 5% NGS overnight at 4°C.
6. Wash in PBS with 5% NGS at 22°C for 30 minutes.
7. Incubate in secondary antibody, biotinylated goat anti-rabbit IgG in PBS with 5% NGS at 22°C for 1 hour, diluted:

1) 1/200, 125 µl/25 ml
2) Per instructions, Vector ABC Elite Kit, 8 drops/25 ml

8. Wash in PBS with 5% NGS at 22°C for 30 minutes.
9. Block endogenous peroxidase activity. Immediately before use, mix 81 ml PBS, 9 ml methanol and 10 ml 30% H2O2. Incubate for 10 minutes at 22°C.
10. Wash in PBS only 1 x 10 minutes at 22°C.
11. Wash in PBS only 1 x 20 minutes at 22°C
12. Wash in PBS with 5% NGS at 22°C for 30 minutes.

Prepare ABC reagent, if using this option.

13. Incubate in:

1) KPL Streptavidin-peroxidase conjugate diluted 1/200 (125 µl/25 ml) in PBS with 5% NGS, 0.1% Tween 20.
2) Vector ABC elite, diluted according to kit instructions for 1 hour at 22°C.

14. Wash in PBS with 5% NGS at 22°C for 10 minutes.
15. Incubate with DAB (2-5 minutes). Stop reaction with PBS wash.
16. Allow to air dry.
17. Wash salts off in ddH2O. Dehydrate, clear and mount on chromalum gelatin coated slides.

Notes:

Paraformaldehyde:
2.76g monobasic
21.45g dibasic heptahydrate
1000 ml ddH2O
40g Paraformaldehyde

PBS (10mM):
0.276 monobasic
2.15g dibasic heptahydrate
100 ml ddH2O
8.76 NaCl/1000 ml
900 ml Isotonic saline + 100 ml mono/dibasic
pH to 7.4

Primary antibody: 17 µl/50 ml PBS with 5% NGS

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