OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-888-267-4436
Fax: +1-301-340-8606
techsupport@origene.com

OriGene Technologies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info-de@origene.com

AP31660SU-N Polyclonal Antibody to PMN - Serum

Alternate names: Polymorphonuclear Leukocytes

Quantity: 1 ml

Host: Rabbit

Format: **State:** Liquid serum

Applications: 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.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody reacts to PMN.
Species: Mouse.
Other species not tested.

Storage: Store the antibody at -20°C.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings: 1. Green et.al., J. Imm. Methods, vol 70: 257, (1984)

Protocols: 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 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