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AP31661SU-N Polyclonal Antibody to PMN - Serum

Alternate names: Polymorphonuclear Leukocytes

Quantity: 2 ml

Background: CLAD51140 is an antiserum directed against rat PMNs (Polymorphonuclear Leukocytes). This antisera is adsorbed repeatedly with red blood cells and lymphocytes
to remove most antibodies to antigens common among different cell types. This adsorption significantly improves cytotoxic specificity.

Host: Rabbit

Format: **State:** Liquid serum

Applications: **Cytotoxic assay.**
Immunohistochemistry on frozen and paraffin sections.
For Cytotoxic Antibodies:
Modified Colorimetric Microtiter Assay 1
Results: Antisera of the above this antibody diluted 1/20 exhibits ›80% cytotoxicity on rat PMNs
Antisera diluted 1/20 exhibits
‹15% cytotoxicity on rat 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 the above this antibody strongly
agglutinates rat PMNs but not lymphocytes at dilutions 1/20 to 1/200
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

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

Storage: Upon receipt, store (in aliquots) at -20°C to -80°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)
2. Andrews et al., Am. J. Physiol., Polymorphonuclear leukocyte infiltration into gastric tissue. 266; 48-54 (1994).

Protocols: Rabbit anti-Rat PMN IHC protocol
(can also be used for mouse sections)
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-rat PMN), AP31661SU-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