

Product datasheet for **AM31860PU-N**

Platelets Rat Monoclonal Antibody [Clone ID: AIP21]

Product data:

Product Type:	Primary Antibodies
Clone Name:	AIP21
Applications:	FC, FN
Recommended Dilution:	Flow Cytometry (See Protocols). Functional Assays.
Reactivity:	Mouse
Host:	Rat
Isotype:	IgM
Clonality:	Monoclonal
Specificity:	This anti-Mouse platelet Monoclonal antibody AM31860PU-N(clone AIP21) detects an unidentified antigen on Mouse platelets that is not identical to CD9, GPIV or integrins. This antigen is also present on B16F10 and KN-3 cells, but not thymocytes or splenocytes. AM31860PU-N induces Mouse platelet aggregation in the absence of plasma components via an FcR-independent mechanism. A dramatic increase in tyrosine phosphorylation of the 52 kDa Shc protein was observed during AIP21-mediated platelet aggregation.
Formulation:	PBS containing 0.02% Sodium Azide as preservative. State: Purified State: Liquid purified IgM fraction.
Concentration:	lot specific
Purification:	Affinity Chromatography.
Conjugation:	Unconjugated
Storage:	Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.



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Background:

Platelets or thrombocytes are the blood cell fragments that are involved in the cellular mechanisms that lead to the formation of blood clots. Low levels or dysfunction predisposes for bleeding, while high levels, although usually asymptomatic, may increase the risk of thrombosis. Like red blood cells, platelets are anuclear (no cell nucleus) and discoid (disc shaped); they measure 1.5 to 3.0 μm in diameter. The body has a very limited reserve of platelets, so they can be rapidly depleted. They contain RNA, a canalicular system, and several different types of granules; lysosomes (containing acid hydrolases), dense bodies (containing ADP, ATP serotonin and calcium) and alpha granules (containing fibrinogen, factor V, vitronectin, thrombospondin and von Willebrand factor), the contents of which are released upon activation of the platelet. These granule contents play an important role in both hemostasis and in the inflammatory response.

Note:

Protocol: **Flow Cytometry Analysis:**

Method:

1. Prepare a cell suspension of mouse platelets in media A.
2. Wash 2 times.
3. Resuspend the cells to a concentration of 2×10^7 cells/ml in media A. Add 50 μl of this suspension to each tube (each tube will then contain 1×10^6 cells, representing 1 test).
4. To each tube, add $\sim 1.0 \mu\text{g}^*$ of AM31860PU-N.
5. Vortex the tubes to ensure thorough mixing of antibody and cells.
6. Incubate the tubes for 30 minutes at 4°C .
7. Wash 2 times at 4°C .
8. Add appropriate amount of FITC anti-Rat IgM secondary antibody (or similar).
9. Incubate the tubes at 4°C for 30-60 minutes.
(It is recommended that the tubes are protected from light since most fluorochromes are light sensitive).
10. Wash 2 times at 4°C in media B.
11. Resuspend the cell pellet in 50 μl ice cold media B.
12. Transfer to suitable tubes for flow cytometric analysis containing 15 μl of propidium iodide at 0.5 mg/ml in PBS. This stains dead cells by intercalating in DNA.

Media:

- A. Phosphate buffered saline (pH 7.2) + 5% normal serum of host species + sodium azide (100 μl of 2M sodium azide in 100 mls).
- B. Phosphate buffered saline (pH 7.2) + 0.5% Bovine serum albumin + sodium azide (100 μl of 2M sodium azide in 100 mls).