

M040-3

Product Information

Catalog Number: M040-3
Clone / Isotype: Xia.G5 / Rat (Wistar) IgG2b
Contents: DyLight 649-labeled immunoglobulin in 20 mM Tris buffer with 137 mM NaCl, 0.5% BSA, and 0.09% (w/v) sodium azide
Size: 1.5 ml / 300 tests

For research use only, not for diagnostic or therapeutic use. This product is no medical device.

Specificity: The Xia.G5 antibody reacts with mouse GPIb α (CD42b), a platelet/megakaryocyte-specific 150 kDa polypeptide that is disulfide-linked with GPIb β (24 kDa) in the membrane. GPIb is part of the GPIb-V-IX complex, the platelet receptor for von Willebrand factor (vWf)^{1,2}. Xia.G5 binds to the N-terminal 45 kDa domain of GPIb α ³, which contains the binding sites for different ligands of the receptor, including vWf, thrombin, and P-selectin¹. GPIb α is proteolytically cleaved (glycocalicin) during platelet activation.

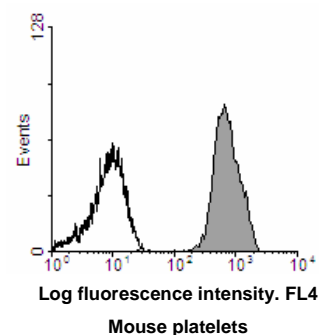
Preparation and Storage: The antibody was purified from hybridoma cell culture supernatant by Protein G-Sepharose chromatography. The antibody was conjugated with DyLight 649 under optimum conditions. The solution is free of unbound fluorescent dye. Store product undiluted at 4°C and avoid prolonged exposure to light. Stable for one year from date of shipment. Do not freeze.

Usage: The antibody preparation is optimized for flow cytometric applications: Use 5 μ l to stain $\sim 10^6$ platelets or $\sim 0.5 \times 10^6$ cells in a recommended volume of 25 μ l. Incubate for 15 minutes at room temperature, stop reaction by addition of 400 μ l PBS and analyze samples within 30 minutes. For immunofluorescent staining of acetone-fixed frozen sections, the appropriate dilution must be determined individually.

Caution: Sodium azide is a reversible inhibitor of oxidative metabolism; therefore, antibody preparations containing this preservative agent must not be used in cell cultures nor injected into animals. Sodium azide may be removed by washing stained cells or plate-bound antibody or dialyzing soluble antibody in sodium azide-free buffer.

Detection of GPIb α on mouse platelets

Mouse blood was diluted 1:20 and 25 μ l of this dilution were stained with 5 μ l control IgG-649 (emfret Analytics, P190-3, *black line*) or Xia.G5-649 (*shaded area*) for 15 min at RT and analyzed directly. Platelets were gated by FSC/SSC characteristics.



- References:**
- Berndt MC, Shen Y, Dopheide SM, et al. (2001) The vascular biology of the glycoprotein Ib-IX-V complex. *Thromb Haemost.* 2001 Jul;86(1):178-88.
 - Bergmeier W, Rackebandt K, Schroder W, Zirngibl H, Nieswandt B. (2000) Structural and functional characterization of the mouse von Willebrand factor receptor GPIb-IX with novel monoclonal antibodies. *Blood.* 95:886-93.
 - emfret Analytics. Unpublished results.