

D200

Product Information

Catalog Number:	D200	
Clones / Isotypes:	JON/A / Rat (Wistar) IgG2b	(1 ml – 200 tests)
	Wug.E9 / Rat (Wistar) IgG1	(1 ml – 200 tests)
Contents:	PE- and FITC-labeled immunoglobulins in 20 mM Tris buffer with 137 mM NaCl, 0.5% BSA and 0.09% (w/v) sodium azide	
Size:	2 x 1.0 ml / 200 tests	

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

This product provides a ready-to-use tool to determine integrin α IIb β 3 (GPIIb/IIIa) activation and α -granule secretion (P-selectin expression) in mouse platelets in a single analysis.

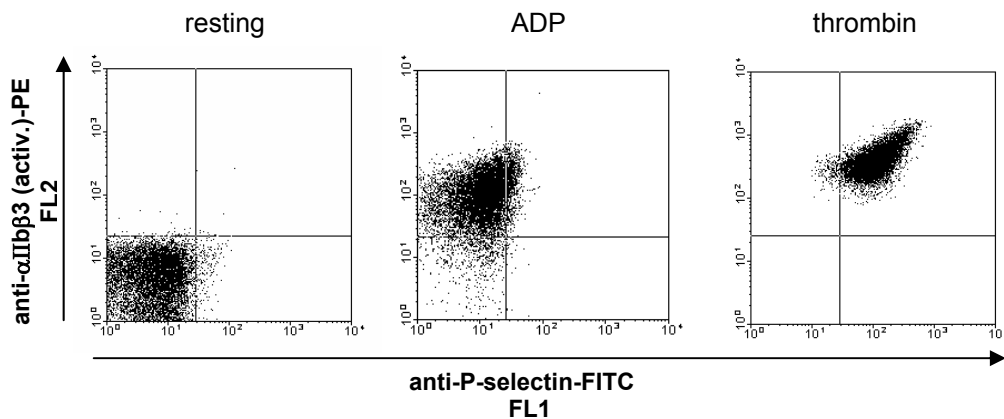
Specificity: The PE-labeled JON/A antibody (emfret # M023-2) selectively binds to the high affinity conformation of mouse integrin α IIb β 3¹ (GPIIb/IIIa, CD41/CD61), a glycoprotein complex consisting of the 135-kDa α IIb chain and the 90-kDa β 3 chain. Integrin α IIb β 3 is a platelet receptor for fibrinogen, von Willebrand factor, fibronectin, and vitronectin, and it mediates platelet adhesion and aggregation². The activation-dependent conformational change in integrin α IIb β 3, and therefore binding of JON/A-PE is dependent on extracellular free calcium¹.

The FITC-labeled Wug.E9 antibody (emfret # M130-1) reacts with mouse P-selectin (CD62P), a 140-kDa single-chain polypeptide belonging to the selectin family of adhesion molecules³. P-selectin is expressed in the α -granules of platelets and in the Weibel-Palade bodies of endothelial cells. Upon cellular activation, P-selectin is translocated to the surface membrane. P-selectin mediates the adhesion of activated platelets to neutrophils and monocytes⁴. The surface expression of P-selectin on platelets is a marker for α -granule secretion.

Preparation and Storage: The antibodies were purified from hybridoma cell culture supernatant by Protein G-Sepharose chromatography. The antibodies were conjugated with FITC or R-Phycoerythrin (PE) under optimum conditions. The solutions are free of unbound fluorophores. Store product undiluted at 4°C and avoid prolonged exposure to light. Stable for one year from date of shipment. Do not freeze. **Please mix equal volumes of Antibody A (JONA-PE) and Antibody B (Wug.E9-FITC) in the mixing tube before use.** The mixture can be stored for several weeks at 4°C.

Usage: The antibody preparation is optimized for flow cytometric applications. It is recommended to use 10 μ l of the mixture of Antibody A and Antibody B to stain $\sim 10^6$ platelets or $\sim 0.5 \times 10^6$ cells in a volume of 25 μ l Tyrode-Hepes buffer containing 1 mM CaCl₂. Agonists should be added together with the staining antibodies. Incubate for 15 min at room temperature, stop reaction by addition of 400 μ l PBS and analyze samples within 30 minutes. Please note that changes in incubation time, buffer conditions, or antibody concentration may influence the quality of the results.

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 activated integrin α IIb β 3 and P-selectin-expression on mouse platelets

10^6 mouse platelets in 25 μ l Tyrode-Hepes buffer (1 mM CaCl_2) were left untreated or stimulated with 10 μ M adenosine diphosphate (ADP) or 0.2 U/ml thrombin and directly stained with 10 μ l of a mixture of Antibody A and Antibody B for 15 min at RT. Samples were filled up with 400 μ l PBS and analyzed directly. Platelets were gated by FSC/SSC characteristics.

Note: ADP is a relatively weak agonist which stimulates (reversible) integrin α IIb β 3 activation but virtually no P-selectin expression. In contrast, thrombin is a very strong platelet agonist which induces strong and irreversible integrin α IIb β 3 activation and P-selectin expression.

References:

1. Bergmeier W, Schulte V, Brockhoff G, Bier U, Zirngibl H, Nieswandt B. (2002) Flow cytometric detection of activated mouse integrin α IIb β 3 with a novel monoclonal antibody. *Cytometry* 1;48:80-6.
2. Phillips DR, Charo IF, Scarborough RM. (1990) GPIIb-IIIa: the responsive integrin. *Cell*. 65(3):359-62.
3. Bird MI, Foster MR, Priest R, et al. (1997) Selectins: physiological and pathophysiological roles. *Biochem Soc Trans*. 25(4):1199-206
4. Bouchard BA, Tracy PB. (2001) Platelets, leukocytes, and coagulation. *Curr Opin Hematol*. 8(5):263-9