Two-color analysis of mouse platelet activation
PE- and FITC- labeled antibodies

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 αIIbβ3 (GPIIb/IIIa) activation and α-granule secretion (P-selectin expression) in mouse platelets in a single analysis.

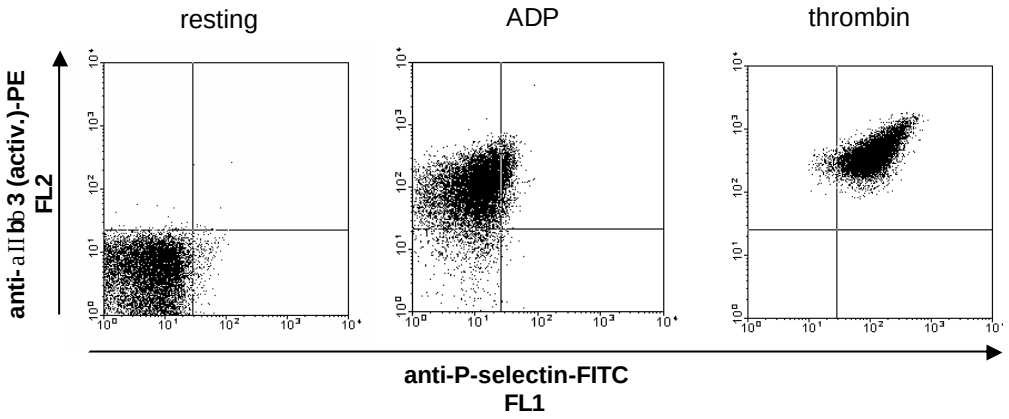
Specificity: The PE-labeled JON/A antibody (emfret # M023-2) selectively binds to the high affinity conformation of mouse integrin αIIbβ3[1] (GPIIb/IIIa, CD41/CD61), a glycoprotein complex consisting of the 135-kDa αIIb chain and the 90-kDa β3 chain. Integrin αIIbβ3 is a platelet receptor for fibrinogen, von Willebrand factor, fibronectin, and vitronectin, and it mediates platelet adhesion and aggregation[2]. The activation-dependent conformational change in integrin αIIbβ3, and therefore binding of JON/A-PE is dependent on extracellular free calcium[1].

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 molecules[3]. 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 monocytes[4]. 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 µl of the mixture of Antibody A and Antibody B to stain ~10^6 platelets or ~$0.5x10^6$ cells in a volume of 25 µl Tyrode-Hepes buffer containing 1 mM $CaCl_2$. Agonists should be added together with the staining antibodies. Incubate for 15 min at room temperature, stop reaction by addition of 400 µl 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 αIIbβ3 and P-selectin-expression on mouse platelets
10^8 mouse platelets in 25 µl Tyrode-Hepes buffer (1 mM $CaCl_2$) were left untreated or stimulated with 10 µM adenosine diphosphate (ADP) or 0.2 U/ml thrombin and directly stained with 10 µl of a mixure of Antibody A and Antibody B for 15 min at RT. Samples were filled up with 400 µl PBS and analyzed directly. Platelets were gated by FSC/SSC characteristics.
Note: ADP is a relatively weak agonist which stimulates (reversible) integrin αIIbβ3 activation but virtually no P-selectin expression. In contrast, thrombin is a very strong platelet agonist which induces strong and irreversible integrin αIIbβ3 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 alphaIIbbeta3 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