Monolith Protocol MO-P-051

Anti-CD42b – CD42b (on intact platelets)

The glycoprotein (GP) Ib-IX-V complex is a vital receptor for the initiation of hemostasis as well as thrombosis. It binds to the multimeric adhesive ligand, von Willebrand factor, found in endothelial cells, megakaryocytes, and platelets. It is essential for regulating platelet adhesion under high shear to sub endothelium, endothelial cells or leukocytes, procoagulant complex assembly on the platelet surface and signaling involving activation of integrins. Its absence or deficiency leads to Bernard-Soulier syndrome. GP1b, also called CD42b is a 145 kDa protein that, together with CD42c, forms a heterodimer of 160 kDa consisting of an α-chain and a β-chain. The different subunits are linked together via a disulfide bridge. CD42c forms a non-covalent complex together with CD42a and CD42d in the membrane of the platelet. The CD42 complex is a receptor of von Willebrand factor and thrombin, which mediate thrombocyte adhesion to subendothelial matrixes, which are exposed during endothelial damage.

antibody – receptor interaction | platelets | whole cell interaction

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A1. Target/Fluorescent Molecule

Anti-CD42b mAb

A2. Molecule Class/Organism

Monoclonal antibody directed against human CD42b
Mouse

A3. Sequence/Formula

N/A

A4. Purification Strategy/Source

Monoclonal Mouse Anti-Human CD42b, Platelet Glycoprotein 1b, Clone AN51
Dako
R7014

A5. Stock Concentration/Stock Buffer

0.6 µM
Buffer containing 1% bovine serum albumin (BSA), 15 mM NaN_3, pH 7.2

A6. Molecular Weight/Extinction Coefficient

150 kDa

A7. Dilution Buffer

Phosphate buffered saline (PBS), pH 7.4

A8. Labeling Strategy

Labeled with RPE (R-Phycoerythrin), Dako

A9. Labeling Procedure

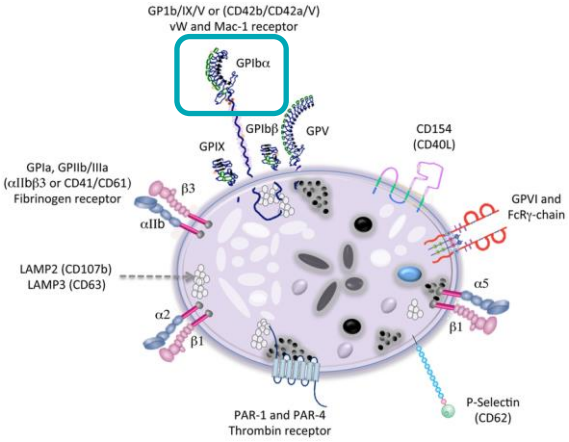
N/A

A10. Labeling Efficiency

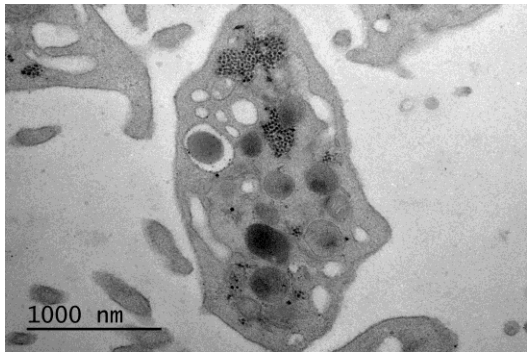
N/A

B1. Ligand/Non-Fluorescent Binding Partner

CD42b α-chain on the surface of platelets
uniprot.org/uniprot/P07359



Receptors present on the surface of human platelets



Electron microscopy picture of a human platelet

B2. Molecule Class/Organism

Receptor/human platelets
Homo sapiens

B3. Sequence/Formula

MPLLLLLLLL PSPLHPHPIC EVSKVASHLE VNCDKRNLTA LPPDLPKDTT ILHLSENLLY TFSLATLMPY TRLTQLNLDR
CELTKLQVDG TLPVLGTLDL SHNQLQSLPL LGQTLPALTV LDVSFNRLTS LPLGALRGLG ELQELYLKGN ELKTLPPGLL
TPTPKLEKLS LANNNLTELP AGLLNGLENL DTLLLQENSL YTIPKGFFGS HLLPFAFLHG NPWLCNCEIL YFRRWLQDNA
ENVYVWKQGV DVKAMTSNVA SVQCDNSDKF PVYKYPGKGC PTLGDEGDTD LYDYYPEEDT EGDKVRATRT VVKFPTKAHT
TPWGLFYSWS TASLDSQMPS SLHPTQESTK EQTTFPPRWT PNFTLHMESI TFSKTPKSTT EPTPSPTTSE PVPEPAPNMT
TLEPTPSPTT PEPTSEPAPS PTTPEPTSEP APSPTTPEPT SEPAPSPTTP EPTPIPTIAT SPTILVSATS LITPKSTFLT
TTKPVSLLES TKKTIPELDQ PPKLRGVLQG HLESSRNDPF LHPDFCCLLP LGFYVLGLFW LLFASVVLIL LLSWVGHVKP
QALDSGQGAA LTTATQTTHL ELQRGRQVTV PRAWLLFLRG SLPTFRSSLF LWVRPNGRVG PLVAGRRPSA LSQGRGQDLL
STVSIRYSGH SL

B4. Purification Strategy/Source

Platelets were isolated according to the following protocol[1]. Blood was collected in 9 mL ACD-A Vacuette® tubes (Greiner Bio-One GmbH, Frickenhausen, Germany). The tubes were centrifuged at 150 × g for 15 minutes to get the platelet rich plasma (PRP). The PRP was collected and supplemented with 0.25 U/mL apyrase and 1 µM prostacyclin (PGI2) and centrifuged again at 480 × g for 20 minutes. After gently replacing the plasma phase with Krebs-Ringer Glucose (KRG; 120.24 mM NaCl, 5.02 mM KCl, 1.24 mM $MgSO_4 \cdot 7H_2O$, 8.47 mM $Na_2HPO_4 \cdot 2H_2O$, 10 mM glucose and 1.73 mM KH_2PO_4, pH 7.3) supplemented with PGI2, the platelet pellet was resuspended in the same buffer. Thereafter the platelets were counted.

To calculate the receptor concentration on the surface of the platelets, the following calculations were performed:

# of platelets: $1.7 \cdot 10^{12}\ L^{-1}$

# of receptors / platelet: $\sim 2.4 \cdot 10^{5}$

Molar concentration: $(1.7 \cdot 10^{12}\ L^{-1} \cdot 2.4 \cdot 10^{5}) / 6.023 \cdot 10^{23}\ mol^{-1} = 0.68 \times 10^{-6}\ M$

B5. Stock Concentration/Stock Buffer

~0.68 µM
Krebs-Ringer Glucose (KRG)

B6. Molecular Weight/Extinction Coefficient

71.5 kDa

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of 0.68 µM CD42b coated platelet (intact cells) into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
2. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
3. Add 10 µL of 15 nM anti-CD42b-PE to each tube from **16** to **1** and mix by pipetting.
4. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

D1. MST System/Capillaries

Monolith NT.115 Blue (NanoTemper Technologies GmbH)
Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

D2. MST Software

MO.Affinity Analysis v2.3 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith/#monolith-software

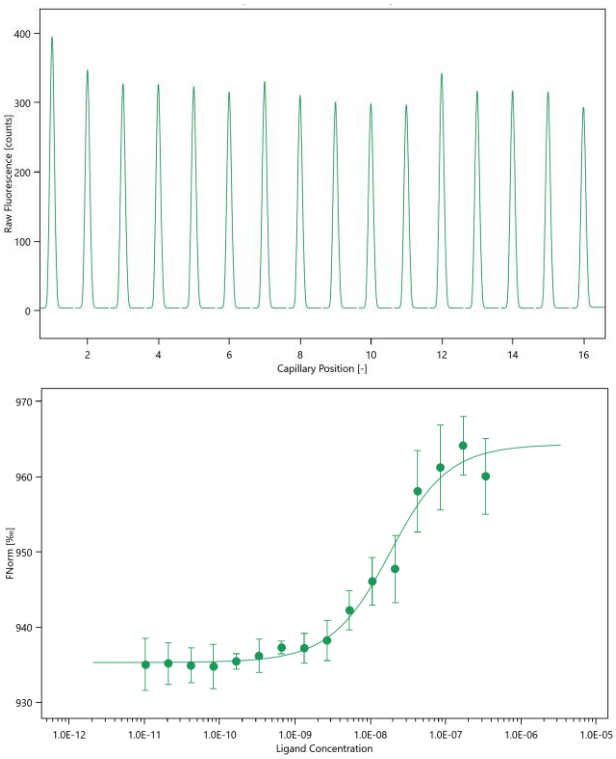
[1] See Macwan et al., Haematologica 2019, 104: 1482-1492 and Maddermann et al., J Biol Chem 267 (1), 364-369 (1992) for more information.

D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

Phosphate buffered saline (PBS)[2], pH 7.4
7.5 nM anti-CD42b-PE | 0.68 µM – 0.01 nM CD42b coated intact platelets | 22°C | medium MST power | 100% excitation power

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

K_d = 14 nM



D5. Reference Results/Supporting Results

N/A[3]

E. Contributors

Ankit Macwan[4], Tomas Lindahl[2], Teresia Hallström[5]

[2] Be aware that detergents may influence the cells.
[3] A RPE-labeled monoclonal isotype control was used as a negative control.
[4] Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden
[5] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com