Simple and sensitive method for the flow cytometric quantitation of platelet associated immunoglobulin.

Reagent kit contains various polyclonal antibodies labelled with the fluorescence dye phycoerythrin (PE): antibodies against total human immunoglobulins, against human IgA, IgM and IgG and against rabbit immunoglobulin as a negative control. The kit also contains anti-CD42a-FITC and further reagents.

Standardized test procedure for the evaluation of thrombocytopenia (low platelet count). The determination of autoantibodies helps to differentiate immune from nonimmune thrombocytopenia.

Quantitative analysis of platelet associated total immunoglobulin for the differential diagnosis of Idiopathic Thrombocytopenic Purpura (Morus Werlhof, ITP).
A. Sample Preparation

A. Isolation of platelet rich plasma (PRP)

2 ml EDTA whole blood

Centrifuge 10 min, 100 x g without brake!

Remove platelet rich plasma (PRP)

Centrifuge 7 min, 700 x g, decant supernatant

Centrifuge 3 x (7 min, 700 x g) decant supernatant

Repeat centrifugation step once

Resuspend PRP in 1.5 ml 1 x BUF WASH

Incubate overnight at 2 - 8°C

Cell Counting: Adjust concentration of thrombocytes to 20,000/µl

B. Measurement

Set FSC and SSC amplifier gains to log mode. Region 1 set on CD42a positive thrombocytes.

C. Data Evaluation

Region 1 is set on CD42a positive thrombocytes. Display FL2 histograms and analyse the Median values. Examples from patient positive for IgG.

2. Cell Labelling

5 x 100 µl à 2 x 10⁶ PLT PRP suspension

+10 ml REAG B per tube

Mix and incubate 1 min at RT

Mix samples: Incubate for 20 min on ice

+2 ml 1 x Washing Buffer

Centrifuge samples 7 min, 700 x g decant supernatant

+20 ml of REAG C to REAG G

+0.5 ml 1 x Washing Buffer

Centrifuge samples 7 min, 700 x g decant supernatant

+2 ml 1 x Washing Buffer

Mix samples: Incubate for 20 min on ice

BUF WASH = Washing Buffer
REAG B = Goat Serum
REAG C to REAG G = different ployclonal antibodies
REAG H = CD42a-FITC

For more information please contact us

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