Application Information Bulletin : Platelet Activation

Flow Cytometric Measurement of CD62P Platelet Activation

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Flow Cytometric Measurement of CD62P Platelet Activation

INTRODUCTION

This assay uses flow cytometry to measure the expression of P-selectin (CD62) on the surface of human platelets in whole blood both prior to and following ex vivo activation. The mobilization of CD62 from intracellular granules to the cell surface occurs rapidly in response to activating stimuli; for this assay the stimulus is ADP. Measurement of CD62P by this method may be used to assess platelet integrity and activation state, or simply to identify platelets within a mixed blood cell population or monitor non-specific activation of platelets introduced by a particular experimental method.

MATERIALS - EQUIPMENT

- Human whole blood collected in BD Vacutainer Buff Na Citrate Cat # 369714

  Perform sample preparation as soon as possible after blood collection to avoid ex vivo activation of platelets
- CD62P / P-Selectin Antibody, PE conjugate (Psel.KO2.3) (Life Technologies #: A16339)
- Adenosine DiPhosphate (ADP) (Chrono-Log Corp #: Chrono-Par reagents, 384)
- Dulbecco's Phosphate Buffered Saline 1x(PBS) (pH 7.4) (0.2 µm filtered) (Gibco #: 14190-144)
- Polypropylene tubes (Fisher Scientific #: 02-681-200)
- Beckman Coulter CytoFLEX*

WORKING SOLUTIONS

CD62P-PE antibody

Prepare 1:32 antibody dilution from stock:

2 µL Ab + 62 µL 1xPBS = 64 µL of 1:32 dilution CD62P-PE antibody

ADP

Prepare 100 µM working solution from 1 mM stock.

3 µL 1 mM ADP + 27 µL 1xPBS = 30 µL of 100 µM ADP

PROCEDURE

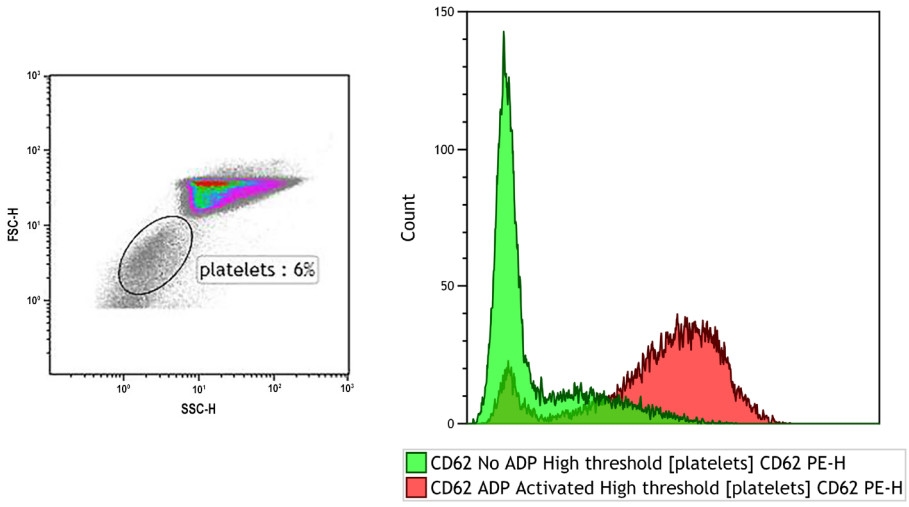
1. Filter 1x PBS (0.2 µm filtered).
2. Prepare the working solutions (see above).
3. Label tubes for flow cytometery (12x75 mm) as follows in duplicate:
    a. No Ab, no ADP
    b. CD62P 1:32, no ADP
    c. CD62P 1:32, + ADP
4. Add reagents according to Table 1 to the appropriate tube

Table 1. Reagents to add to tubes

	No Ab, no ADP (tubes #1 and #4)	CD62P 1:32, no ADP (tubes #2 and #5)	CD62P 1:32 + ADP (tubes #3 and #6)
PBS	34 µL PBS + 11 µL PBS	34 µL PBS+ 6 µL PBS	34 µL PBS
Antibody	-	5 µL 1:32 CD62P	5 µL 1:32 CD62P
ADP	-	-	6 µL ADP
sample	5 µL whole blood	5 µL whole blood	5 µL whole blood

5. Incubate all tubes for 30 minutes at room temperature in the dark.
6. Make up volume in each tube to 800 µL (by adding 750 µL filtered 1x PBS) before flow cytometry.
7. Acquire data as soon as possible

RESULTS



Overlay of platelets stained with CD62P-PE untreated (green) and treated with ADP (red).

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