Creighton UNIVERSITY

Staining Protocol for Platelet Activation Studies

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Equipment:

ACD Vacutainer blood collection tubes
12x75mm disposable polystyrene test tubes
Micropipettor with tips

Reagents:

ADP Stock @ $2x10^{-4}$ Molar
RGDS Stock @ 10 mg/ml
1% formalin, cold
Monoclonal antibodies and isotype controls:

Antibodies	*Isotypes*
Mouse anti-human CD41 (PE)	Mouse isotype control (PE)
Mouse anti-human CD62p (APC)	Mouse isotype control (APC)
Mouse anti-PAC-1 (FITC)	Mouse isotype control (FITC)

Procedure:

Blood collection:

1) Collect ~2ml of blood aseptically by venipuncture into any type of Vacutainer. Discard as it contains activated platelets.
2) Collect blood aseptically by venipuncture into ACD Vacutainer. Perform activation of platelets within 10 minutes.

Activation: *(must be performed within 10 minutes of blood collection)*

1) Put 50 µl of ADP solution into a 12x75mm test tube.
2) Add 450 µl of blood. Mix gently.
3) Incubate 2 minutes at room temperature.
4) Stain or fix immediately.

Staining:

1) Label 6 12x75mm test tubes for each patient as follows:
   - Resting – 3 color
   - Resting – Isotypes
   - Resting – RGDS
   - Activated – 3 color
   - Activated – Isotypes
   - Activated – RGDS
2) Add antibodies to the test tubes, then add the cells. Mix gently.
3) Incubate for 15-20 minutes at room temperature in the dark.
4) Add 1ml of cold 1% formalin to each tube and mix well.
5) Store stained and fixed cells refrigerated in the dark for at least 30 minutes, but not more than 24 hours.

Overview of Platelet Staining Protocol

	Tube					
	Resting 3 Color	**Resting Isotypes**	**Resting RGDS**	**Activated 3 Color**	**Activated Isotypes**	**Activated RGDS**
Cells	5 µl resting	5 µl resting	5 µl resting	5 µl activated	5 µl activated	5 µl activated
CD41 (PE)	5 µl		5 µl	5 µl		5 µl
CD62p (APC)	10 µl		10 µl	10 µl		10 µl
PAC-1 (FITC)	10 µl			10 µl		10 µl
PE Isotype		5 µl			5 µl	
APC Isotype		10 µl			10 µl	
FITC Isotype		10 µl			10 µl	
RGDS			10 µl			10 µl