Jun 27, 2024

Platelet purification and coating of plates for adhesion assays

DOI
dx.doi.org/10.17504/protocols.io.n92ld8wb8v5b/v1

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Protocol Citation: Samantha King 2024. Platelet purification and coating of plates for adhesion assays . **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ld8wb8v5b/v1

Protocol status: Working
We use this protocol and it's working

Created: June 27, 2024

Last Modified: June 27, 2024

Protocol Integer ID: 102535

Keywords: Platelet preparation

Funders Acknowledgement:
American Heart Association
Grant ID: 19TPA34760750

Abstract

This is the protocol we use to prepare fixed platelets from human blood and coat wells ready for adhesion assays.

Guidelines

We typically have greater success when using at least 180 mls of blood. When using smaller volumes of blood we get a smaller number of platelets than expected

Materials

*Store all buffers at 4^{0}C

ACD
(Acid citrate dextrose solution)
39mM citric acid, 75mM sodium citrate,
135mM dextrose, pH 7.4.
Before use, warm up to room temperature
and adjust pH if necessary

HEP
Buffer
140mM NaCl, 2.7mM KCl, 3.8mM HEPES, 5mM
EGTA, pH 7.4
Before use, warm up to room temperature
and adjust pH if necessary.

Wash
buffer
10mM sodium citrate, 150mM NaCl, 1 mM EDTA,
1% (w/v) dextrose, pH 7.4.
Before use, warm up to room temperature
and adjust pH if necessary.

Safety warnings

Relevant biosafety protocols must be followed.

1 Blood from healthy donors was collected in 1/6th volume of acid citrate dextrose solution (ACD). Mix gently.

2 Blood was spun down for 20 min at 200 x g, deaccelerating step without brake.

3 Platelet rich plasma was collected (top yellow layer) and mixed (inverting the tube) 1:1 with HEP buffer.

4 Platelets were collected by centrifugation at 800 x g for 20 min without brake. At this step supernatant was removed without disturbing the pellet.

5 Platelets were washed twice with 1 ml platelet wash buffer as indicated: rinse the platelet pellet with
platelet wash buffer (without resuspension in order to avoid unnecessary platelet activation) by gently adding wash buffer and removing it slowly with a pipette. Repeat once. After the last wash, resuspend the pellet in 3 ml wash buffer.

6 Platelets were fixed with 1% paraformaldehyde for 10 minutes, incubate platelets at room temperature for 10 minutes.

7 Pellet platelets by centrifuging at 700 x g for 10 minutes (no brake) and wash them with 10 ml PBS pH
7.4 Repeat once.

8 After the second wash, resuspend platelets in PBS pH 7.4 (If we start with 180 mls of blood we typically use 10 mls here). Count platelets and then adjust the volume of PBS to have $1x10^7$ cells/mL.

9 Coat flat bottom 96-well plate wells with 100 µL of platelets ($1x10^7$). We coat triplicate wells for each sample and for each strain coat three control wells with 100 µL of 1% BSA.

10 Incubate the plate for 1h at 37°C.

11 Aspirate liquid and wash wells by adding 120 µL of PBS to each well. Remove liquid again and finally add 120 µL of PBS to each well.

12 The plates are covered in parafilm and keep in the cold room for further use. They keep at least one month