May 07, 2018

Human platelet isolation

DOI
dx.doi.org/10.17504/protocols.io.pymdpu6

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.pymdpu6

Protocol Citation: Allen Bennett 2018. Human platelet isolation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.pymdpu6

Protocol status: In development
We are still developing and optimizing this protocol

Created: May 07, 2018

Last Modified: May 07, 2018

Protocol Integer ID: 12013

Abstract

Platelets are the primary cellular mediator of thrombosis. This protocol allows for the preparation of washed human platelets. These platelets can then be stimulated or cultured for various purposes.

Guidelines

Handle platelets gently at all steps to avoid activating them

Materials

MATERIALS

Tyrodes Buffer **Boston Bioproducts** **Catalog #**PY-921

PGI2 (10mM) **Santa Cruz Biotechnology** **Catalog #**61849-14-7

Safety warnings

Human blood is a biosafety hazard. Be familiar and have approval to work under BSL-2 conditions.

Before start

Prepare Tyrode's Buffer, or use Tyrode's buffer from a commerical source

1 Use IV butterfly set and draw blood into sodium citrate vaccutainers

2 Centrifuge at 1200 rpm for 15 minutes at room temperature

3 Transfer plasma top fraction to a new tube

4 Dilute plasma with an equal volume of Tyrodes buffer

5 Add PGI2 to a final concentration of 10uM

6 Centifuge at 2800 rpm for 5 minutes, decant the supernatant and gently resuspend the platelet pellet into Tyrode's buffer

7 Dilute the platelets 1:20 in fresh Tyrode's buffer for downstream applications such as flow cytometry, or lyse pellet directly for ELISA or western blotting.