Human platelet isolation

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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.

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Guidelines

Handle platelets gently at all steps to avoid activating them

Before start

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

Materials

Tyrodes Buffer PY-921 by Boston Bioproducts
PGI2 (10mM) 61849-14-7 by Santa Cruz Biotechnology

Protocol

Step 1.

Use IV butterfly set and draw blood into sodium citrate vaccutainers

Step 2.

Centrifuge at 1200 rpm for 15 minutes at room temperature

Step 3.

Transfer plasma top fraction to a new tube

Step 4.

Dilute plasma with an equal volume of Tyrodes buffer

Step 5.

Add PGI2 to a final concentration of 10uM

Step 6.

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

Step 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.

Warnings

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