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Isolation of Platelets Protocol

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We use this protocol and it's working

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Abstract

This protocol details the isolation of platelets protocol.

Guidelines

The protocol needs prior approval by the users' Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s) as applicable.

Materials

Required buffers:

Platelet wash buffer

	A	B
	Sodium citrate	10 mM
	NaCl	150 mM
	EDTA	1 mM
	dextrose, pH 7.4	1% (w/v)

Before use, warm up to room temperature and adjust pH if necessary.

HEP buffer (HEP refers to HEPES in the buffer)

	A	B
	NaCl	140 mM
	KCl	2.7 mM
	HEPES	3.8 mM
	EGTA, pH 7.4	5 mM

Before use, warm up to room temperature and adjust pH if necessary.

Tyrode's buffer

	A	B
	NaCl	134 mM
	NaHCO ₃	12 mM
	KCl	2.9 mM
	Na ₂ HPO ₄	0.34 mM
	MgCl ₂	1 mM
	HEPES	10 mM

Add 5 mM glucose and freshly added BSA (3 mg/ml). pH 7.4

Depending on experiment, warm buffer up to room temperature or keep on ice before use.

Platelets inhibitors:

- P5515-1MG Prostaglandin E1 (Molecular formula: C₂₀H₃₄O₅; Molecular weight: 354.5; 1mg total; 1 μM final concentration):

1. **Preparation Instructions:** It is recommended that a stock solution of 10 mg/ml in ethanol be prepared and that aliquots be further diluted with 0.1 M phosphate buffer to achieve the desired concentration. Make dilutions slowly to avoid crystallization of the prostaglandin.
2. It is reported that prostaglandin E1 is soluble at approximately 7.5 ug/ml in water at 25°C.
3. **Prostaglandin E1 in ethanol at 10 mg/ml is stable for longer than 6 months at -20°C.**
4. Prostaglandins are generally unstable in aqueous acid or alkaline solutions.
5. Prostaglandin E1 has a maximum stability between pH 6-7.2.
6. When aqueous solutions are frozen, the prostaglandin may precipitate but it will usually redissolve on shaking or if necessary with brief ultrasonication.
7. The use of plastic tubes should be avoided when working with dilute aqueous concentrations of prostaglandins. If the tube's surface is rough and irregular on a microscale, a hydrophobic interaction similar to the interaction in reverse phase chromatography takes place with some loss of material due to attachment to the surface. Teflon is suitable to use since it has a smooth surface.

- A6535-200UN Apyrase (0.2 U/ml final concentration):

1. **Preparation Instructions:** This product is soluble in water (1 mg/mL).

	A	B
	NP40	2X platelet lysis buffer 2%
	HEPES	30 mM
	NaCl	150 mM
	EDTA, pH 7.4	2 mM

For preparation of a lysate of quiescent platelets, add instead an ice-cold 1:1 mixture of Tyrode's buffer and 2X platelet lysis buffer, including protease inhibitors.



Safety warnings

- ❗ The protocol needs prior approval by the users' Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s) as applicable.

Before start

The protocol needs prior approval by the users' Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s) as applicable.

Blood Fractionation

45m

- 1 The first step of this procedure includes obtaining the whole blood and preparing platelet-rich plasma (PRP) by centrifugation. The centrifugation conditions can vary widely (e.g., from 800 x g, 00:05:00 to 100 x g, 00:20:00) but give consistently good separations.
 - A phlebotomist should draw blood into a Becton Dickinson Vacutainer® (containing ACD or EDTA) or into a plastic syringe containing 1/10 volume of CPD buffer.
 - The volume of blood needed depends on the experiment. A volume of 40–45 ml blood results in average $1\text{--}3 \times 10^9$ platelets.
 - The platelet number can vary depending on the individual the blood is drawn from.
We use 10–15mL.

25m



- 2 Mix gently during and after blood collection by slowly inverting the Vacutainer® or syringe.



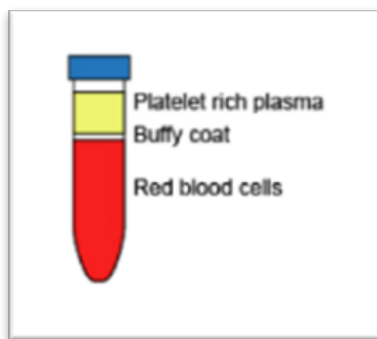
- 3 If a Vacutainer® is not being used, transfer blood into a plastic tube suitable for centrifugation by using a plastic transfer pipette (wide orifice).

- 4 Spin at 200 x g, 00:20:00 at Room temperature (with no brake applied).

20m



- 5 After the spin, three distinct layers can be observed:



Bottom layer: Red blood cells (accounting for 50–80% of the total volume)

Middle layer: Very thin band of white blood cells (also called “buffy coat”)

**Top layer:** Straw-colored PRP

Platelets obtained from the blood bank (30% plasma, 70% PAS) or platelet-rich plasma PRP (protocol):

40m

6 Platelet Isolation

- Transfer two-thirds of the PRP (from the blood fractionation described above) into a new plastic tube using a transfer pipette (wide orifice) without disturbing the buffy coat layer, in order to avoid contamination.

7 Add **HEP buffer** at a 1:1 ratio (v/v). Include prostaglandin E1 (PGE1, 1 μ M final concentration) to prevent platelet activation. (e.g. if you have 100mL of PRP, use a 50ml Falcon® and do the process 4 times; ratio 1:1 means 25ml PRP and 25 ml HEP buffer).



8 Mix very gently by inverting the tube slowly.



9 Spin at 100 x g for 00:15:00 – 00:20:00 at Room temperature (with no brake applied) to pellet contaminating red and white blood cells.

20m



10 Transfer the **supernatant** into a new plastic tube using a transfer pipette (wide orifice) (could be like the pipette used to do the pH adjustments, with wide orifice to avoid platelets to clog the pipette tube).



11 Pellet platelets by centrifugation at 800 x g for 00:15:00 – 00:20:00 at Room temperature (with no brake applied). **Discard the supernatant.**

20m



12 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. (Repeat until pink color is gone, the purpose of this is to clean the pellet and get rid of red blood cells).

13 Carefully and slowly resuspend the pellet in Tyrode's buffer containing 5 millimolar (mM) glucose and freshly added BSA (3 μ L) using a transfer pipette (wide orifice). To prevent platelet activation add PGE1 (1 micromolar (μ M)) and/or apyrase (0.2 U/ml final concentration). Release the buffer slowly along the tube wall and minimize the amount of agitation.

- If the platelets need to be activated in subsequent experiments, omit the addition of PGE1 and apyrase at this step.



- **For preparation of a lysate of quiescent platelets, add instead an ice-cold 1:1 mixture of Tyrode's buffer and 2X platelet lysis buffer, including protease inhibitors.**

A	B
NP40	2X platelet lysis buffer 2%
HEPES	30 mM
NaCl	150 mM
EDTA, pH 7.4	2 mM

- 14 Count the platelets (e.g. by using a hemocytometer, semi-automated Coulter-type counters or an electronic particle analyzer).
- 15 Adjust the platelet concentration as needed with Tyrode's buffer. A common concentration used for various platelet studies (e.g. platelet activation, aggregation and adhesion assays, western blotting and immunocytochemistry/immunofluorescence) is $1-3 \times 10^8$ platelets/ml.