

# Isolation of human platelets from whole blood

This protocol describes the isolation of human platelets from whole blood.

To prevent the activation of platelets during the procedure, strong mechanical forces (e.g. fast pipetting, vigorous shaking) should be avoided. In addition, the platelet inhibitors indicated in the protocol can be used, but other inhibitors exist as well. It is the researcher's choice to select inhibitors that are most suitable for their studies and experimental goals.

Examples of applications for isolated platelets are:

Western blotting, flow cytometry, ELISA and immunocytochemistry/immunofluorescence to investigate cellular and signaling processes within the platelets. Platelet aggregation and adhesion upon stimulation with various agents can be studied as well as the release of granule components upon activation.

## Reagents

Unless platelets are used in tissue culture experiments, the reagents do not need to be sterile. Store and use all buffers at 4°C, unless indicated otherwise.

### **ACD buffer (acid-citrate-dextrose)**

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

### **CPD buffer (citrate-phosphate-dextrose)**

16 mM citric acid, 90 mM sodium citrate, 16 mM NaH<sub>2</sub>PO<sub>4</sub>, 142 mM dextrose, pH 7.4.  
Before use, warm up to room temperature and adjust pH if necessary.

### **Platelet wash buffer**

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

### **HEP buffer (HEP refers to HEPES in the buffer)**

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

### **Tyrode's buffer**

134 mM NaCl, 12 mM NaHCO<sub>3</sub>, 2.9 mM KCl, 0.34 mM Na<sub>2</sub>HPO<sub>4</sub>, 1 mM MgCl<sub>2</sub>, 10 mM HEPES, pH 7.4.  
Depending on experiment, warm buffer up to room temperature or keep on ice before use.

### **2X platelet lysis buffer**

2% NP40, 30 mM HEPES, 150 mM NaCl, 2 mM EDTA, pH 7.4.

## **Blood collection notes**

- Many drugs are known to interfere with platelet studies and platelet function. It is important to ensure that potential blood donors have not taken such medication (e.g. anti-histamines, aspirin, non-steroidal anti-inflammatory medication) two weeks prior to the blood draw.
- Platelets are recommended to be handled and stored in polypropylene, polyethylene or polycarbonate lab ware.

- Blood and intact platelets should preferably be handled at room temperature or 37°C.
- Blood is a bodily fluid and should be considered biohazardous. Protective equipment should be worn at all times and blood should be disposed of according to the regulations of the researcher's institution.

## Blood fractionation

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 for 5 min to 100 x g for 20 min), but give consistently good separations.

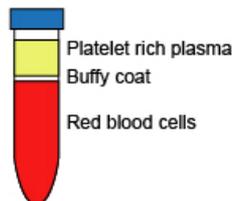
A phlebotomist should draw blood into a Becton Dickinson Vacutainer® (containing ACD, yellow cap) or into a plastic syringe containing 1/10 volume of CPD buffer. The volume of blood needed depends on the experiment. A volume of 40–45 ml blood results in average  $1\text{--}3 \times 10^9$  platelets. The platelet number can vary depending on the individual the blood is drawn from.

1. Mix gently during and after blood collection by slowly inverting the Vacutainer® or syringe.
2. If a Vacutainer® is not being used, transfer blood into a plastic tube suitable for centrifugation by using a plastic transfer pipette (wide orifice).
3. Spin at 200 x g for 20 min at room temperature (with no brake applied).
4. 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



**Fig 1. Schematic representation of blood constituents**

## Platelet isolation

1. 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.
2. Add HEP buffer at 1:1 ratio (v/v). Include prostaglandin E1 (PGE1, 1  $\mu\text{M}$  final concentration) to prevent platelet activation.
3. Mix very gently by inverting the tube slowly.
4. Spin at 100 x g for 15–20 min at room temperature (with no brake applied) to pellet contaminating red and white blood cells.
5. Transfer the supernatant into new plastic tube using a transfer pipette (wide orifice).
6. Pellet platelets by centrifugation at 800 x g for 15–20 min at room temperature (with no brake applied). Discard the supernatant.
7. 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.
8. Carefully and slowly resuspend the pellet in Tyrode's buffer containing 5 mM glucose and freshly added BSA (3 mg/ml) using a transfer pipette (wide orifice). To prevent platelet activation add PGE1 (1  $\mu\text{M}$ ) 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.***

9. Count the platelets (e.g. by using a hemocytometer, semi-automated Coulter-type counters or an electronic particle analyzer).
10. 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.