ab289839 – Human Platelet Isolation Kit

For isolation of platelets from human whole blood.
For research use only - not intended for diagnostic use.

Storage and Stability

All components in this kit are shipped on blue ice and are suitable for storage at 4°C, unless reconstituted. Upon receipt, immediately store kit at 4°C in the dark, except Cell Dye II which should be stored at -20°C after it has been reconstituted. Individual components may be stored at alternative temperatures as shown in the table below. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Temperature (on receipt)	Storage Temperature (post reconstitution)
Gradient Dilution Buffer I	110 mL	4°C	4°C
Density Gradient Media II	25 mL	4°C	4°C
Platelet Storage Buffer	2 x 50 mL	4°C	4°C
BSA Solution	5 mL	4°C	4°C
Cell Dye II	1 vial	-20°C	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- DMSO.
- PBS.
- 15 mL conical tubes (polypropylene or polyethylene).
- Human blood 5 mL collected in EDTA or ACD anticoagulant.
- Red Blood Cell Lysis Buffer (if desired).
- Multi-well plates, sterile.

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Gradient Dilution Buffer I, Density Gradient Media II, and Platelet Storage Buffer: Use in a sterile environment to prevent contamination of isolated platelets. Bring to room temperature (RT) before use and mix well.

Cell Dye II: Reconstitute vial in 100 µL DMSO. Light sensitive, do not expose to intense light. Store at -20°C, protected from light.

Assay Protocol

Platelet Isolation and Viability Assay Protocol (5 ml total volume of whole blood/isolation):

- Work in a sterile environment. Use universal precautions when handling blood products and human body fluids.
- Handle blood and platelet samples carefully to avoid activation of the platelets.
- This kit has sufficient reagents to isolate platelets from 5 mL samples.

1. **Sample Preparation:** Prepare the following solutions to create the density gradient:
   - **a.** Density Barrier Solution (DBS, 1.072 g/mL). Add 5.0 volumes of Density Gradient Media II (DGM) to 22 volumes of Gradient Dilution Buffer I (GDB). For 5mL of DBS, add 0.925 mL DGM to 4.07 mL GDB. Mix well by inverting the mixture several times.
   - **b.** Blood: Gently invert 4-5 times to mix.
   - **c.** Platelet Storage Buffer: If you are using the buffer to store the isolated platelets, add 200 µL BSA Solution to 0.8 mL of Platelet Storage Buffer.

     Δ Notes:
     - *a) If either of the reagents is opened outside the hood, filter sterilization of the reagent is recommended.*
     - *b) If Platelet Storage Buffer is used for washing the platelets,* BSA Solution *is not necessary.*

2. **Density Gradient Preparation:**
   - **a.** Add 5 mL of DBS at the bottom of a 15 mL conical tube. Then, slowly and carefully layer 5 mL of blood on top of DBS.

     Δ Notes:
     - a) Due to stickiness of the blood components, always layer blood on top of DBS.
     - b) Blood volume < 5 mL can be used but the minimum volume of DBS should be ~5 mL. For large volumes of blood, ratio of blood to DBS should 1:1.
   - **b.** Centrifuge at 350 x g for 15 min at ambient temperature (21-25 ˚C). Set the centrifuge to STOP without braking.

     Δ Note: We recommend using a centrifuge with a swing-bucket rotor and no brakes to prevent disturbing the layers.

3. **Harvesting the Platelet Layer:**
   - **a.** Remove the conical tube. There should be 3+ layers: The top plasma layer, the middle DBS layer containing platelets, and the bottom layer containing RBCs and leukocytes. There will be a slight gradient (or change in opacity) in the upper 2-3 ml of the DBS layer because of the platelets distribution. There may be a cloudy, more concentrated layer of platelets at the plasma interface.
   - **b.** Carefully remove the plasma layer and discard it.
   - **c.** Remove the cloudy band of platelets, if visible, near the plasma/DBS interface.
   - **d.** Collect the remaining top 80-90% of the DBS band. Avoid taking the last 0.5-1 mL near the blood cell/DBS interface because it contains numerous RBCs and possibly leukocytes.

4. **Determination of Live Platelets:**
   - **a.** Prepare a 1:10 dilution of the Cell Dye II by adding 2 µL of Cell Dye II to 18 µL of PBS in a tube. Mix thoroughly.
   - **b.** Dilute the platelets by adding 5 µL of platelets into 95 µL of 1x PBS. Mix well by gently pipetting up and down.
   - **c.** Add 5 µL of diluted Cell Dye II solution to 45 µL of diluted platelets.
   - **d.** Gently pipette the platelets up and down ten times. Incubate for 20 min at 37°C in the dark. The sample is ready for analysis.
   - **e.** Repeat gently mixing of the platelet sample by pipetting up and down at least ten times. Load the required volume in the counting chamber of a cell counter slide or 10 µl on an Hemocytometer.
   - **f.** Wait 5-10 min to allow the platelets to settle in the chamber and then count the platelets.
   - **g.** For automated cell counter, enter the dilution factor (1:22.2).
   - **h.** For Hemocytometer, record the total platelet count using a 40x objective lens in Bright-field by counting the four corner squares and the center square, which are 5 squares of the 25 total in the hemocytometer's large central square.

The platelet count is: (Platelets counted in 5 squares) * (Dilution Factor) / Area x Depth = (Platelets counted) * 1100

- **i.** Viability: Within the same Region of Interest (ROI) in view, reduce the white light, open the fluorescent lamp shutter and view the cells in FITC/TRITC filter to count the fluorescent cells in the same area. If a small amount of incidental white light illuminates the Hemocytometer, the grid will be visible allowing the viewer to see the same ROI as was visible in the fluorescent light. Live platelets will fluoresce green. Dead platelets will not fluoresce. Tally the number of green platelets to complete the calculation using the equation:

  % Live platelets = (number of fluorescent platelets (green) / total number of platelets in bright field view) x 100

  Δ Note: Contaminating RBCs will not fluoresce. WBC will be much larger and will fluoresce, if they are viable.

5. **Wash Step (Optional):**
   - **a.** Dilute the collected platelets with equal volumes of Platelet Storage Buffer. Centrifuge at 2500 x g for 10 min at RT. Allow the platelets to rest for at least 15 min after centrifugation.
   - **b.** Carefully remove the supernatant and gently resuspend the platelets using a wide bore tube or pipet.
   - **c.** Resuspend at a concentration in Platelet Storage Buffer with 2 mg/mL BSA Solution or in any desired buffer or reagent.

6. **Storage of Platelets (Optional):**
   - **a.** Allow the platelets to rest for at least 15 min after the isolation or the wash step. Resuspend either in Platelet Storage Buffer or in any other media or buffer.
     Δ Note: Platelets are best stored with constant agitation at RT (20-24°C or 6875°F). However, because RT is conducive to bacterial and fungal growth, it is very important that all the reagents are sterile and the isolation steps are performed in a sterile environment.

Technical Support

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