FOR RESEARCH USE ONLY!

Human Platelet Isolation Kit

rev 01/21

(Catalog # K2071-25; 25 isolations; Store at -20 °C)

I. Introduction:

Platelets, also known as thrombocytes are small (2-4 µm in diameter), lens-shaped, anuclear cells found in the blood. They are produced in the bone marrow by the fragmentation of megakaryocytes. After fragmentation, they migrate into the circulation where they make up 4-7% of all blood cells. Like many other types of blood cells, platelets have mutiple functions. Their primary role is in blood clotting. They also have an important role in innate immune response, inflammatory processes, and destruction of pathogens. Platelets can be found in two distinct forms namely unactivated and activated. The average lifespan of a platelet in blood is 3-10 days. **BioVision's human Platelet Isolation Kit** allows the *in vitro* isolation of intact, viable, unactivated platelets. The kit enables the high recovery of platelets of (≥ 1 x10^7platelets/ml) yielding approximately 80% of the total platelets present in 1 ml of whole blood. The viability stain included in the kit is used to identify living platelets indicating that > 90% of the platelets in the isolated fraction is viable. The isolated fraction contains > 95% platelets and has minimal contamination of red blood cells and leukocytes.

II. Applications:

- Isolation of platelets from whole blood.
- Determination of platelet viability, purity, and quality.
- Studying platelet activation and characterization of platelet morphology.
- *In vitro* assays to evaluate primary platelet functions such as clotting, chemokine release, adhesion, chemotaxis etc.
- Studying platelet surface proteins.

III. Sample Types:

- Fresh whole blood collected < 8 hrs prior to platelet isolation, with EDTA or citrate anti-coagulants.

Notes:

1. For isolating healthy platelets, blood donor should not be taking aspirin for at least 48 hr or anti-platelet medications such as Plavix or Brilinta.
2. Platelets are fragile structures. Thus, it requires gentle handling and treatment to retain their *in vivo* properties and to prevent activation.

IV. Kit Contents:

Components	K2071-25	Cap Code	Part Number
Gradient Dilution Buffer	110 ml	NM	K2071-25-1
Density Gradient Media	25 ml	NM	K2071-25-2
Platelet Storage Media	50 ml x 2	NM	K2071-25-3
BSA Solution	5 ml	NM	K2071-25-4
Viability Stain	1 vial	Green	K2071-25-5

V. User Supplied Reagents and Equipment:

- Laminar flow hood to keep the reagents sterile
- DMSO
- PBS
- 15 ml conical tubes (polypropylene or polyethylene)
- Centrifuge with Swinging bucket Rotor
- Fluorescent Microscope with a dual FITC/TRITC Filter
- Hemocytometer
- Human blood 5 ml collected in EDTA or ACD anticoagulant
- Rocker platform if planning on storing platelets >24 hours
- Red Blood Cell Lysis Buffer (if desired)
- Multi-well plates, sterile

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge vials prior to opening. Read the entire protocol before performing the assay.

- **Gradient Dilution Buffer, Density Gradient Media, and Platelet Storage Media:** Use in a sterile environment to prevent contamination of isolated platelets. Bring to room temperature (RT) before use and mix well.
- **Viability Staining (lyophilized):** Reconstitute vial in 100 µl DMSO. **Light sensitive, do not expose to intense light.** Store at -20°C, protected from light.

VII. 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 **(DGM)** to 22 volumes of Gradient Dilution Buffer **(GDB).** For 5ml of DBS, add 0.925 ml DGM to 4.07 ml GDB. Mix well by inverting the mixture several times.

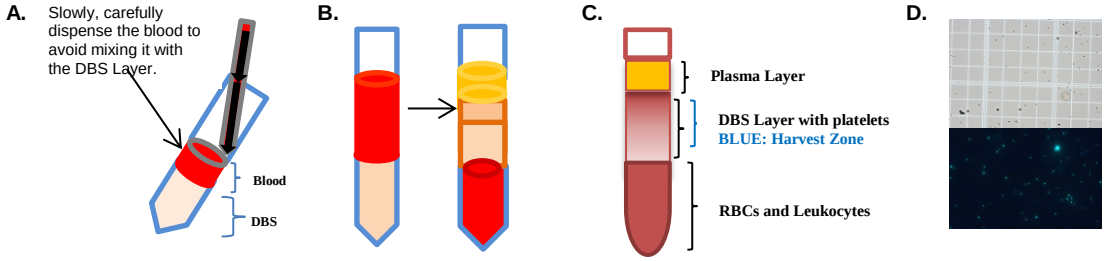
   b. **Blood:** Gently invert 4-5 times to mix.

   c. **Platelet Storage Buffer:** If you are using the buffer to store the isolated platelets, add 200 µl BSA to 0.8 ml of Platelet Storage Buffer.

   Notes:

   1. If either of the reagents is opened outside the hood, filter sterilization of the reagent is recommended.
   2. If Platelet Storage Buffer is used for washing the platelets, BSA 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 **(Figure A).**
     Notes:
     i. Due to stickiness of the blood components, always layer blood on top of DBS.
     ii. 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.
     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 **(Figure B).**
   - **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 **(Figure C).**
4. **Determination of Live Platelets:**
   - **a.** Prepare a 1:10 dilution of the Viability Stain by adding 2 µl of Viability Stain 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 Viability Stain 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
     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. Carefully remove the supernatant and gently resuspend the platelets using a wide bore tube or pipet. Resuspend at a concentration in Platelet Storage Buffer with 2 mg/ml BSA 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.
     Note: Platelets are best stored with constant agitation at RT (20-24 ˚C or 68-75 ˚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.



Figures A. Illustration of a conical tube held at a 45° angle while blood is layered on top of DBS. **B.** Layers of DBS and the whole blood before and after centrifugation, showing the separation of layers in the conical tube. **C.** Separation of three layers (plasma, DBS with platelets and RBCs/leukocytes). **D.** Bright-field image of platelets and image from a fluorescent microscope with FITC/TRITC filters of same ROI showing live (green) platelets.

VIII. RELATED PRODUCTS:

Human Whole Blood Polymorphonuclear Cell Isolation Kit (K483)
Human Peripheral Blood Mononuclear Cell Isolation and Viability Kit (K541)
Platelet Activating Factor (PAF) ELISA Kit (E4631)
Human Whole Blood Monocyte Isolation Kit (K933
Cell Surface Protein Isolation Kit (K295)
Red Blood Cell Lysis Buffer (5831)