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Technical Datasheet

HiPer® Lymphocyte Isolation and Staining Teaching Kit

Product Code: CCK060

Contents

1. About the kit

HiPer® Lymphocyte Isolation and Staining Teaching Kit has been developed for teaching isolation of lymphocytes from whole blood and visualization of the separated lymphocytes by Giemsa staining

The kit is sufficient for separation and staining of lymphocytes from 5 blood samples.

2. Kit contents and storage:

On receipt, remove the contents of the kit and place them in appropriate storage locations as per recommended storage temperature.

Contents		Quantity	Store at
Code	Description		
CCK060(A)	Heparinized blood collection tube	5 No.	2 - 8°C
CCK060(B)	Lymphocyte Separation Medium	5ml	2 - 8°C
CCK060(C)	Centrifuge tubes, 15ml Sterile, Conical bottom	10 No.	15-30°C
CCK060(D)	RPMI-1640	10ml	2 - 8°C
CCK060(E)	Tissue Culture Flask, 12.5cm^2, Non-treated, Vented cap	5 No.	15-30°C
CCK060(F)	Glass slides Fine polished edges, Frosted One End Both Sides	5 No.	15-30°C
CCK060(G)	Giemsa Satin Solution 20X	1ml	15-30°C
CCK060(H)	Dulbecco's Phosphate Buffered Saline 1X	25ml	15-30°C
CCK060(I)	Trypan Blue Solution 0.5% in DPBS	0.5ml	15-30°C

• Quantities supplied in excess to compensate operational losses

3. Materials required but not provided in the kit

3.1 Blood collection accessories

- Needle
- Syringe
- Tourniquet
- Cotton
- Spirit solution

3.2 Equipments

- Laminar air flow hood
- Compound light microscope with 100X oil immersion objective
- Inverted microscope
- Centrifuge

3.3 Consumables

- Micropipettes
- Serological pipettes
- Pipette aid (LA692)
- Disposable gloves
- Lab coat
- sopropanol spray
- Tissue paper

3.4 Chemicals (for preparation of Carnoy's fixative)

- Methanol
- Glacial acetic acid

4. Aseptic techniques and good cell culture practices

a. Use Personal Protective Equipment (PPE), (laboratory coat, gloves and eye protection) at all times while working in a cell culture lab. Use head caps to cover hair.

b. PPE for tissue culture facility should be kept separate from PPE worn in general laboratory environment.

c. Before starting tissue culture work switch on the UV light in the biosafety cabinet for 15-20 minutes.

d. Keep all the work surfaces free from clutter.

e. All reagent and media bottles should be labeled correctly with name and date of preparation and should be kept at recommended storage temperatures.

f. Clean the working area of the laminar air flow hood with 70% isopropanol.

g. Prior to starting work all reagent and media bottles, pipettes, tip boxes should be sprayed with 70% isopropanol.

h. Arrange the work station in such a way that you have an easy access to all the items and a wide clear space in the centre of the bench.

i. Keep all the reagents and media bottles to the left hand side of work station and the consumables and discard beaker to the right hand side of work station for efficient working.

j. While working do not contaminate the gloves by touching anything outside the cabinet (especially face and hair). In case they become contaminated then, respray with 70% isopropanol before proceeding.

k. In case of any spillage while working, mop up immediately and swab the area with 70% isopropanol.

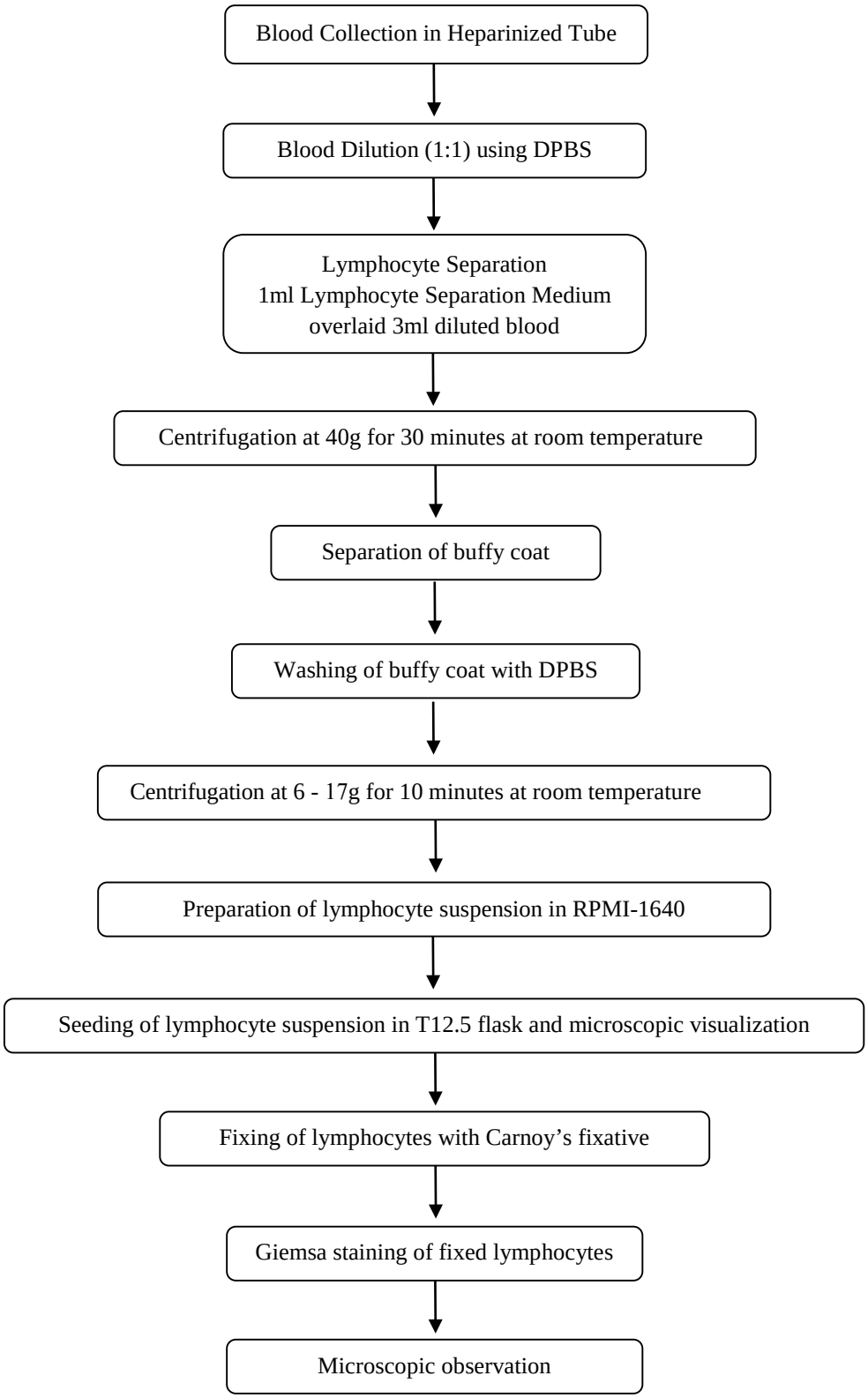
l. Avoid rapid movement within and immediately outside the cabinet. Slow movement will allow the air within the cabinet to circulate properly.

m. Avoid speaking, sneezing and coughing while working in the cabinet to prevent the contamination.

n. Pipette tips, waste reagents and waste medium should be discarded carefully into a separate discard beaker.

o. Once the work is finished, clear the working area and clean with 70% isopropanol.

5. Directions for use



5.1. Blood collection and dilution

1. Equilibrate heparin containing tube to room temperature.
2. Draw 2ml peripheral blood from a healthy volunteer by venipuncture and collect it in the heparinized tube.
3. Cap the tube tightly and mix gently by inverting 2-3 times to avoid coagulation.
   ***Note**: Do not shake vigorously*.
4. Aseptically add 2ml of Dulbecco's Phosphate Buffered Saline [CCK060(H)] to 2ml blood.
5. Mix gently by pipetting up and down.

5.2. Lymphocyte separation

1. Aseptically add 1ml lymphocyte separation medium [CCK060(B)] to 15ml centrifuge tube [CCK060(C)].
2. Overlay 3ml of diluted blood on the lymphocyte separation medium.
   Note: Add the blood drop by drop along the wall of the tube. This will prevent mixing of the blood with separation medium. Quality of the separation depends on a sharp interphase between the blood and lymphocyte separation medium.
3. Centrifuge the tube at 40g for 30 minutes at 15 – 30ºC.
   Note: Refer nomogram on Page No. 5 for conversion of 'g' force to rpm.

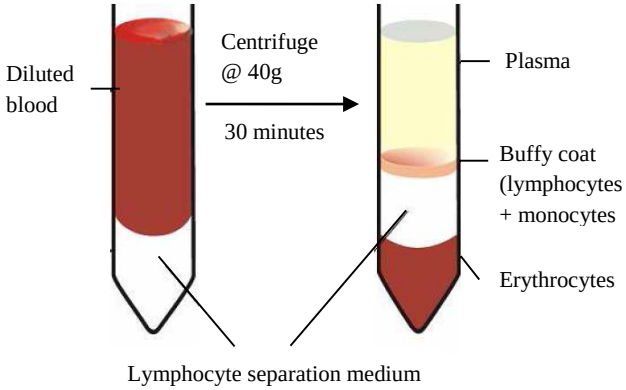


Fig 1: Separation of blood cells by density gradient centrifugation

4. As shown in Fig. 1, after separation the uppermost layer consists of blood plasma. Buffy coat contaminating lymphocytes and monocytes lies below the plasma. A layer of lymphocyte separation medium lies below the buffy coat. Erythrocytes get sedimented at the bottom.
5. Carefully remove the plasma with the help of pipette.
6. Keep small amount of plasma on the buffy coat.
7. Remove buffy coat along with half of the layer of lymphocyte separation medium below it.
8. Collect it in a separate centrifuge tube.

5.3. Seeding and microscopic observation of lymphocytes

1. Add 1ml Dulbecco's Phosphate Buffered Saline [CCK060(H)] to the tube containing buffy coat.
2. Centrifuge at 6 – 17g for 10 minutes at room temperature. This step removes traces of lymphocyte separation medium and platelets.
3. Discard the supernatant and add 1ml of Dulbecco's Phosphate Buffered Saline to the pellet.
4. Suspend the cells by pipetting gently up and down to prepare uniform suspension of lymphocytes.
5. Aseptically add 2ml RPMI-1640 to a 12.5cm^2 flask [CCK060(E)].
6. Add 200µl of lymphocyte suspension to the flask and swirl it gently to spread the suspension uniformly.
7. Observe the flask under inverted microscope at 10X. Lymphocytes are non-adherent round shaped cells.

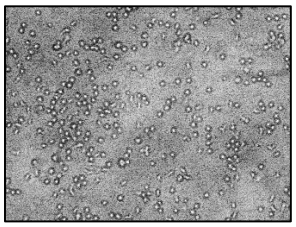


Fig 2: Peripheral blood lymphocytes (10X)

5.4. Lymphocyte staining

1. Prepare Carnoy's fixative by adding 1ml glacial acetic acid to 3 ml methanol. Mix well.
2. Mix the lymphocyte suspension thoroughly and evenly with the help of pipette.
3. Aseptically place a small drop of suspension on a clean slide [CCK060(F)].
4. Make thin smear and allow it to air dry.
5. Add a few drops of Carnoy's fixative on the slide covering the smear completely.
6. Allow the cells to get fixed for 10 minutes at room temperature.

7. Decant the fixative and air dry the slide.
8. Prepare working Giemsa stain solution by diluting concentrated Giemsa stain solution [CCK060(G)] in distilled water in 1:20 ratio just prior to use.
9. Add 1X Giemsa stain solution on the slide covering the entire smear.
10. Stain for 20 minutes at room temperature.
11. Decant the stain solution and gently wash the stained slides with distilled water to remove excess stain.
12. Observe under the microscope at different magnifications.

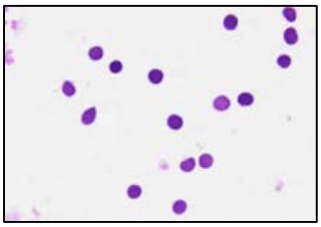


Fig 3: Lymphocytes stained with Giemsa (10X)

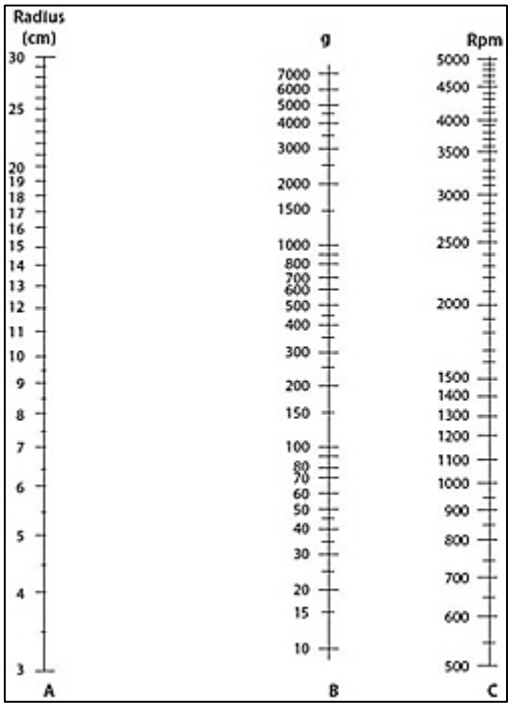
6. Storage and Shelf Life:

Store CCK060(A), (B) and (D) at 2 – 8ºC away from bright light.

Store CCK060(C), (E), (F), (G), (H) and (I) at room temperature away from bright light.

Use before expiry date given on the product label.

Nomogram for determining relative centrifuge forces



1. Measure the radius (cm) from the center of the centrifuge spindle to the end of the test tube carrier. Mark this value on scale A in the above nomogram.
2. Mark the required relative centrifugal force on scale B (e.g. 40g).
3. With a ruler, draw a straight line between points on columns A and B, extending it to intersect column C. The reading on column C is the rpm setting for your centrifuge machine.