Buffy Coat

From 500mL of Whole Blood
For research use only

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Product Description

Whole blood-derived buffy coat products are rich in white blood cells and platelets and are a good source of cells for in vitro research applications. Each buffy coat is suspended in roughly 50 mL of plasma and contains approximately 2 billion leukocytes/unit. The white blood cell (leukocyte) populations in buffy coat products are very diverse (Figure 1), therefore further processing and characterization are typically required for most research applications.

Product Source

Buffy coats are manufactured from health blood donors that have been qualified using the AABB uniform donor history questionnaire and have been tested for relevant communicable diseases required by FDA.

Product Processing

Buffy Coat products are manufactured from 500 mL whole blood units collected in sodium citrate, dextrose, and citric acid (CPD) anticoagulant. These products are red cell-reduced and contain plasma, platelets and mixed leukocyte populations such as granulocytes, lymphocytes, and monocytes.

Product Storage

Use buffy coat product immediately or store at 4^{o}C for up to 24 hours. It is recommended to warm the product to room temperature for 30-60 minutes before processing through Ficoll-Paque density gradient medium.

Population in buffy coat	% range in buffy coat	Range x10^{6} in buffy coat
Total Leukocytes		~2000
Granulocytes	35 to 80	700 to 1600
Mononuclear cells	20 to 65	400 to 1300
Lymphocytes	14 to 47	280 to 940
CD3+ T cells	7 to 24	140 to 480
CD4+ T cells	4 to 20	80 to 400
CD8+ T cells	2 to 11	40 to 220
B cells	1 to 7	20 to 140
NK cells	1 to 6	20 to 120
CD34+ HPC	0.03 to 0.09	0.6 to 1.8
Monocytes	2 to 12	40 to 240

Figure 1: Predicted range of leukocyte sub-population counts in whole blood-derived buffy coat products. The count range of each leukocyte population is shown, based on 500 mL whole blood source product and reported % distribution ranges of leukocyte subpopulations in whole blood (listed). Ranges are approximate and actual numbers will vary from donor-to-donor.

Recommended Purification of Mononuclear Cells from Buffy Coat Products

Materials

Room Temp PBS without Mg^{2+}/Ca^{2+}
Room temperature Ficoll-Paque Density Gradient Medium
Wash buffer (PBS + 5mM EDTA + 2% human serum)
50 mL conical tubes
Serological pipets
Swing bucket, tabletop centrifuge
Media specific for desired downstream research applications
Hemocytometer or automated cell counter

Protocol

Cells inside buffy coat products are sterile. To maintain sterility, perform all harvesting steps inside a biological safety cabinet, practice sterile technique, and use only sterile supplies and media.

1. Remove product from bag and place into 50 mL conical tubes, 25 mL/tube maximum. Warm product to room temperature if stored at 4^{o}C.
2. Dilute product 1:1 with PBS by doubling the volume of buffy coat in each tube.

3. Place 15 mL of Ficoll into clean 50 mL conical tubes, one each for 35 mL of diluted product you wish to process.
4. Carefully layer 35 mL of the diluted buffy coat on top of the Ficoll layer, taking extreme care to not break the plane of the Ficoll and cause mixing of the two components.
5. Centrifuge at 2200 RCF, 20 minutes, 20^{o}C with break OFF. Start the centrifuge.
    a. If temp is too cold or too hot, separation of mononuclear and polymorphonuclear cells will not occur properly.
    b. If the break is left on, mixing will occur between the Ficoll and mononuclear cells when the centrifuge stops.
6. Carefully remove the tubes from the centrifuge. Using a serological pipet, remove the white interface between the Ficoll and media/plasma layers. Transfer the interface to a clean 50 mL tube. Use 1 tube for each Ficoll tube used.
7. Wash the cells by filling the tubes with Wash Buffer and centrifuging for 10 minutes at 1500 RCF, 20 ^{o}C, break ON. Repeat wash.
8. Pool cells into a single tube and wash one more time by filling the tube with PBS and centrifuging.
9. Resuspend cells in desired media. Remove a sample and count the enriched mononuclear cells.

Warning

This product is composed of human-derived materials. Always wear appropriate personal protective equipment when handling this product and treat it as potentially infectious, using Universal Precautions, regardless of the results of infectious disease testing.

Limitations and Publications

This product is for research use only and not for use in humans, for further manufacture, or resale. Nothing produced directly from this product may be sold. When publishing scientific results obtained using this product, acknowledge supplier as Bio-Sharing.org.