

Frequencies of Cell Types in Human Peripheral Blood

CELL TYPE	FREQUENCIES ¹ (X 10 ⁶ cells/mL)	PERCENT	STEMCELL CELL ISOLATION KITS (CATALOG #)		
			SOURCE	SELECTION METHOD	
				NEGATIVE ²	POSITIVE ³
Erythrocytes	3800 – 6200	93 – 96% of blood cells	PBMC		18352 (GlyA)
Platelets	140 – 450	4 – 7% of blood cells			
Leukocytes	4.1 – 10.9	0.1 – 0.2% of blood cells	Whole Blood	07806	
Lymphocytes	1.1 – 3.5	14 – 47% of leukocytes	Whole Blood	19961HLA ⁴ 15263	18684 (CD3/CD19)
T Cells	0.54 – 1.79	7 – 24% of leukocytes	Whole Blood	19951HLA 15061	18081 (CD3) 18687 (CD2)
			PBMC ⁵	19051	18051 (CD3) 18657 (CD2)
CD4 ⁺ T Cells	0.30 – 1.50	4 – 20% of leukocytes	Whole Blood	15062	18082 (CD4)
			PBMC	19052	18052 (CD4)
Naïve CD4 ⁺ T Cells	0.08 – 0.76	1 – 10% of leukocytes	PBMC	19155	
Memory CD4 ⁺ T Cells	0.25 – 0.81	3 – 11% of leukocytes	PBMC	19157	
Regulatory T Cells (CD4 ⁺ CD25 ⁺)	0.007 – 0.052	0.1 – 0.7% of leukocytes	Whole Blood		15861 (CD4 ⁺ CD127 ^{low} CD25 ⁺) 15862 (CD4 ⁺ CD25 ⁺) 15864 (CD4 ⁺ CD127 ^{low} CD49d ⁺ CD25 ⁺)
			PBMC	19231 (CD4 ⁺ CD127 ^{low}) 19232 (CD4 ⁺ CD127 ^{low} CD49d ⁺)	18062 (CD4 ⁺ CD25 ⁺)
CD8 ⁺ T Cells	0.14 – 0.82	2 – 11% of leukocytes	Whole Blood	15063	18083 (CD8)
			PBMC	19053	18053 (CD8)
Naïve CD8 ⁺ T cells	0.03 – 0.21	0.4 – 2.6% of leukocytes	PBMC	19158	
Memory CD8 ⁺ T Cells	– ⁷	1 – 7% of leukocytes	PBMC	19159	
B Cells	0.07 – 0.53	1 – 7% of leukocytes	Whole Blood	15064 19954HLA	18084 (CD19) 18685 (CD20) 18184HLA (CD19/CD20)
			PBMC	19054 19154 ⁶	18054 (CD19) 18454HLA (CD19/CD20)
Naïve B cells	0.05 – 0.37	0.7 – 4.9% of leukocytes	PBMC	19254	
Memory B cells	– ⁷	0.2 – 1.7% of leukocytes	PBMC		18164
Plasma Cells	– ⁷	0.2 – 2.0% of leukocytes in bone marrow	Whole Blood	15169	18387 (CD138)
			PBMC or Bone Marrow		18357 (CD138)
NK Cells	0.08 – 0.43	1 – 6% of leukocytes	Whole Blood	15065	18687 (CD2)
			Buffy Coat		18085 (CD56)
			PBMC	19055	18055 (CD56) 18657 (CD2)
Myeloid Cells	3.9 – 6.5	53 – 86% of leukocytes	Whole Blood	15272HLA	18287 (CD33) 18683 (CD33/CD66b) 18681 (CD15)
			PBMC		18257 (CD33) 18653 (CD33/CD66b)
Dendritic Cells	0.02 – 0.06	0.3 – 0.9% of leukocytes	PBMC	19251	
Plasmacytoid Dendritic Cells	0.01 – 0.04	0.2 – 0.6% of leukocytes	PBMC	19062	
Monocytes	0.20 – 0.90	2 – 12% of leukocytes	Whole Blood	15068	
			Buffy Coat		18088 (CD14)
			PBMC	19058 ⁸ 19059	18058 (CD14)
Granulocytes	2.13 – 6.35	35 – 80% of leukocytes	Whole Blood		18682 (CD66b)
Neutrophils	2.09 – 5.97	30 – 80% of leukocytes	PBMC	19257	
Eosinophils	0.03 – 0.30	0 – 7% of leukocytes	PBMC	19256	
Basophils	0.01 – 0.08	0 – 2% of leukocytes	PBMC	19069	
Hematopoietic Stem and Progenitor Cells (CD34 ⁺)	0.001 – 0.007	0.03 – 0.09% of leukocytes	Mobilized PBMC or Bone Marrow		18056 (CD34)
			Whole Blood		18086 (CD34)
			Cord Blood	19057 15276	18096 (CD34)
			Bone Marrow	19056 15027	

1. Cell type frequencies will vary from donor to donor. 2. Negative selection labels unwanted cells to isolate desired untouched cells. 3. Positive selection labels and isolates desired cell types. 4. Optimized for HLA analysis. 5. Peripheral Blood Mononuclear Cells. 6. Without CD43 Depletion. 7. Frequency of cells not available in blood. 8. Without CD16 Depletion.

■ EasySep®/RoboSep® Immunomagnetic Isolation ■ RosetteSep® Immunodensity Isolation

Isolate immune and stem/progenitor cells quickly and easily from virtually any source. We offer manual and fully automated immunomagnetic column-free cell separation (EasySep® or RoboSep®), and immunodensity cell separation (RosetteSep®), which have been proven to give highly pure cells that are immediately available for a variety of downstream applications.

Protocols for Processing Whole Blood

Preparing a Nucleated Cell Fraction with HetaSep™

HetaSep™ (Catalog #07806) can be used to isolate nucleated cells from a whole blood sample by removing over 95% of the red blood cells (RBC). Erythrocytes aggregate and rapidly sediment while the nucleated cells remain untouched in the suspension. The nucleated cell-rich supernatant can be harvested and used for downstream cell separations.

- Collect whole blood in a blood collection tube containing heparin or ACD, not EDTA.
- Add 1 part HetaSep™ to 5 parts blood and mix well (see Table 1 for the recommended tube size).
- A) Let the sample sit at room temperature or in a 37°C incubator until the RBC interface is at approximately 50% of the total volume.
OR
B) Centrifuge the sample at room temperature and 90 x g with the brake off (see Table 2 for the recommended time). Remove from the centrifuge and leave undisturbed at room temperature for 10 minutes.
- Harvest the leukocyte-rich plasma layer into a fresh 50 mL tube.
- Top up the harvested fraction with at least 4-fold additional fresh buffer and centrifuge at room temperature and 120 x g for 10 minutes with the brake off.
- Carefully remove the supernatant.
- Optional: Lyse the remaining RBCs with ammonium chloride solution (Catalog #07800).
- Resuspend cells in the recommended medium.

For more information, please refer to the HetaSep™ Product Information Sheet or contact techsupport@stemcell.com.

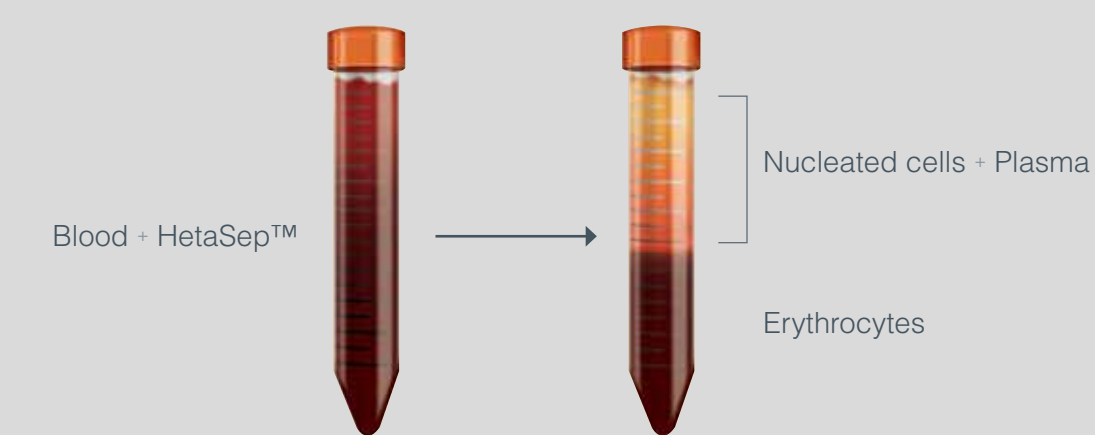
TABLE 1: TUBE SIZE RECOMMENDATIONS

WHOLE BLOOD VOLUME (mL)	RECOMMENDED TUBE SIZE
1 – 4	Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352058)
5 – 10	Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) or Corning® 15 mL Centrifuge Tube (Corning Incorporated, Catalog #430053)

TABLE 2: CENTRIFUGE TIMES BASED ON SAMPLE AGE

START VOLUME BLOOD* (mL)	TUBE SIZE (mL)	SPIN TIME FOR FRESH BLOOD (MINUTES)	SPIN TIME FOR 24 HOUR OLD BLOOD (MINUTES)	SPIN TIME FOR 48 HOUR OLD BLOOD (MINUTES)
2	5	1	1	2
3	5	1	1	4
4	5	2	2	5
10	14	5	5	7

*Start volume refers to volume of blood before HetaSep™ addition.



Preparing a Mononuclear Cell Fraction using Ficoll-Paque™ PLUS

- Mix Ficoll-Paque™ PLUS (Catalog #07957) thoroughly before use.
- Add Ficoll-Paque™ PLUS to an empty tube (see Table 3).
- Dilute the blood 2X with PBS + 2% FBS (see Table 3).
- Layer the blood on top of Ficoll-Paque™ PLUS, being careful to minimize mixing of the blood with the Ficoll-Paque™ PLUS.
- Centrifuge at room temperature at 400 x g for 30 minutes with the brake off.
- Remove and discard the upper plasma layer.
- Remove and retain the mononuclear cell layer at the plasma-Ficoll-Paque™ PLUS interface.
- Wash the mononuclear cells once with PBS + 2% FBS.

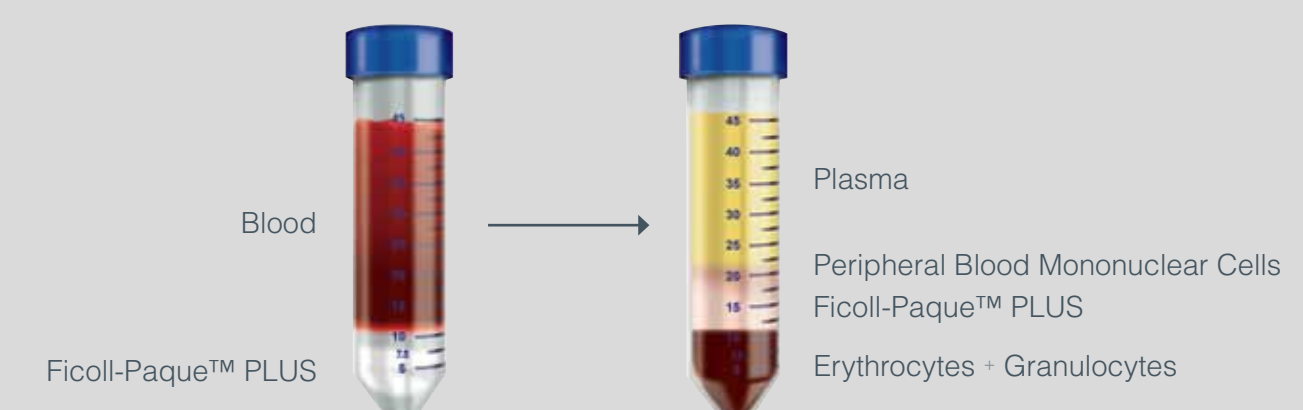
Note: Ficoll-Paque™ PLUS, blood and medium should remain at room temperature for optimum results.

For more information, please refer to the Ficoll-Paque™ PLUS Product Information Sheet or contact techsupport@stemcell.com.

TABLE 3: RECOMMENDED VOLUMES AND TUBE SIZES

BLOOD (mL)	PBS + 2% FBS (mL)	FICOLL-PAQUE™ PLUS VOLUME (mL)	TUBE SIZE (mL)
1	1	1.5	5
2	2	3	14
3	3	3	14
4	4	4	14
5	5	10	50
10	10	15	50
15	15	15	50

Note: We recommend these volumes as guidelines; alternate Ficoll-Paque™ PLUS procedures may be used.



Preparing a Buffy Coat Fraction

- Collect whole blood in a heparinized blood collection tube.
- Add 1 part PBS + 2% FBS and 1 mM EDTA (Ca⁺⁺ and Mg⁺⁺ free) to 1 part fresh whole blood.
- Centrifuge the sample at room temperature and 200 x g for 10 minutes with the brake off.
- Remove the concentrated leukocyte band (this is the buffy coat), and a small portion of the plasma and concentrated RBCs.
- If proceeding with cell separation, transfer a maximum of 4.5 mL of buffy coat to a 14 mL polystyrene tube.

Many EasySep® Whole Blood Kits can be used on buffy coat. For more information, please contact techsupport@stemcell.com.

Conversion of g to RPM

To convert g to rpm, use the following formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-3}) \times \text{radius}}}$$

RCF = relative centrifugal force (g)
RPM = centrifuge speed in revolutions per minute
radius = radius of rotor in cm

For a demonstration of the HetaSep™ or Ficoll-Paque™ PLUS techniques, please view our videos at www.stemcell.com.

Ficoll-Paque™ PLUS is a trademark of GE Healthcare Ltd.



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