Preparing a Nucleated Cell Fraction from Whole Blood with HetaSep™

Separation Principle

RBC aggregation agents such as HetaSep™ increase the RBC sedimentation rate by increasing the effective size of the cells through formation of aggregates, or rouleaux. Because nucleated cells settle at a lower rate, a compact pellet consisting mainly of RBC is formed rapidly in the presence of HetaSep™, while the nucleated cells remain suspended in the supernatant.

Enriching for nucleated cells from whole blood can speed downstream immunomagnetic cell isolation. Leukocyte-rich plasma can be prepared from peripheral blood samples by sedimentation of red blood cells (RBC) using HetaSep™ by either gravity sedimentation or centrifugation. An interface forms between the RBC fraction and the RBC-depleted (nucleated cell-rich) fraction as the RBCs sediment through the HetaSep™ solution. Approximately 99% RBC depletion is attained if the nucleated cell-rich fraction is removed carefully.

Note: The age of the blood sample impacts how fast and to what extent the RBCs sediment. Accordingly, the interface between the plasma fraction and the RBC fraction may be less distinct in older samples. Haemolysis also makes visualization of the interface less obvious.

Gravity Sedimentation Protocol

1. Based on blood sample volume, select an appropriately sized tube (Table 1).
2. Add 1 part HetaSep™ solution to 5 parts blood. Mix well. If using a blood bag, add HetaSep™ directly to the bag and mix.
3. Allow sample to settle until the plasma:RBC interface is at approximately 50% of the total volume. Placing the tube in a 37 ° C incubator for this step will increase the sedimentation rate.
4. Harvest the leukocyte-rich plasma layer and place in a 50 mL tube. Up to 5 - 10% of the initial RBCs may not have sedimented and thus may still remain in this fraction. This is expected.
5. Wash this fraction once with at least a four-fold volume of appropriate medium. This may require several tubes. Perform a slow spin to remove platelets by centrifuging at 120 x g for 10 minutes at room temperature (15 - 25 ° C) with no brake.(¢ to RPM Converter)
6. If excessive platelet contamination is expected, repeat this wash step.
7. Remove supernatant carefully and resuspend the cells in a small volume (typically the nucleated cells from 10 mL of blood would be finally resuspended in 0.5 - 1.0 mL of medium).
8. Optional: Any residual RBCs may be lysed with Ammonium Chloride Solution, if desired.

Table 1. Tube Size Recommendations

Volume	Recommended Tube Size
1 - 4 mL	Falcon™ 5 mL polystyrene round-bottom tubes (Becton-Dickinson, Catalog #352058)
5 - 10 mL	Falcon™ 14 mL round-bottom tubes (e.g. Becton-Dickinson, Catalog #352057)

	OR 15 mL conical tubes (e.g. Corning, Catalog #430053)
> 20 mL	BD Falcon™ 50 mL Conical Tubes (BD, Catalog #352070)

Centrifugation Protocol

Centrifugation may be used to accelerate the sedimentation process.

1. Based on blood sample volume, select an appropriately sized tube (Table 1).
2. Add 1 part HetaSep™ to 5 parts whole blood. Mix well.
3. Centrifuge sample at room temperature (15 - 25 ° C) at 90 xg with the brake off according to Table 2.
4. Remove sample from centrifuge and allow to sit undisturbed at room temperature for 10 minutes. This will allow further sedimentation of the RBC and will improve recovery of the nucleated cells.
5. Harvest the leukocyte-rich supernatant into a fresh 50 mL tube. Up to 5 - 10% of the initial RBCs may not have sedimented and thus may still remain in this fraction. This is expected.
6. Wash this fraction once with at least a four-fold volume of appropriate medium. This may require several tubes. Perform a slow spin to remove platelets by centrifuging at 120 x g for 10 minutes at room temperature (15 - 25 ° C) with no brake.(G to RPM Converter)
7. If excessive platelet contamination is expected, repeat this wash step.
8. Remove supernatant carefully and resuspend the cells in a small volume (typically the nucleated cells from 10 mL of blood would be finally resuspended in 0.5 to 1.0 mL of medium).
9. Optional: Any residual RBCs may be lysed with Ammonium Chloride Solution, if desired.

Table 2. Centrifuge Times Based on Sample Age

		Centrifuge Time (minutes)		
Start Volume of Blood* (mL)	Tube Size (mL)	Fresh Blood	24 hr Old Blood	48 hr Old Blood
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 prior to HetaSep™ addition

Note: Contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com for centrifugation speeds and times if processing blood in a 50 mL tube.

References

1. Regidor C, Posada M, Monteagudo D, Garaulet C, Somolinos N, Fores R, Briz M, Fernandez MN: Umbilical cord blood banking for unrelated transplantation: evaluation of cell separation and storage methods. Exp Hematol 27: 380-385, 1999
2. Rubinstein P, Dobrila L, Rosenfield RE, Adamson JW, Migliaccio G, Migliaccio AT, Taylor PE, Stevens CE: Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc Natl Acad Sci USA 92: 10119-10122, 1995