HetaSep™

For depletion of red blood cells from fresh blood samples and isolation of nucleated cells

Catalog #07806	20 mL
Catalog #07906	100 mL

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Product Description

HetaSep™ is an erythrocyte aggregation agent used to quickly separate nucleated cells from red blood cells (RBCs) in whole blood. Aggregated erythrocytes settle much faster than dispersed cells. By controlling the settling time and/or centrifugation speed, the majority of nucleated cells are recovered in the supernatant. Approximately 95 - 99% RBC depletion is attained if the nucleated cell-rich fraction is removed carefully. HetaSep™ contains 6% w/v hetastarch.

SEPARATION PRINCIPLE

RBC-aggregating 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 RBCs is formed rapidly in the presence of HetaSep™, while the nucleated cells remain suspended in the supernatant.

Properties

Storage: Store at 15 - 25°C. HetaSep™ may be stored at 2 - 8°C; ensure that the solution warms up to room temperature (15 - 25°C) and then invert bottle to mix contents prior to use. Protect from direct light.

Shelf Life: Stable until expiry date (EXP) on label.

Directions for Use

Leukocyte-rich plasma can be prepared from peripheral blood samples by sedimentation of RBCs through HetaSep™ using either gravity sedimentation or centrifugation as outlined below.

NOTE: If using HetaSep™ in conjunction with an EasySep™ negative selection kit, use the HetaSep™ procedure recommended on the EasySep™ Product Information Sheet.

GRAVITY SEDIMENTATION

Gravity sedimentation is a simple and reliable method of RBC depletion. A defined 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.

1. Select an appropriately sized tube.
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 (e.g. Catalog #38010). Wash this fraction once with at least a 4-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 the brake off.

   NOTE: If excessive platelet contamination is expected, repeat this wash step.
5. Carefully remove supernatant and resuspend 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).
6. Optional: Residual RBCs may be lysed with Ammonium Chloride Solution (Catalog #07800).

CENTRIFUGATION

Centrifugation may be used to accelerate the sedimentation process.

1. Based on blood sample volume, select an appropriately sized tube according to Table 1.

Table 1. Recommended Tube Sizes for Centrifugation

VOLUME OF WHOLE BLOOD	RECOMMENDED TUBE SIZE
1 - 4 mL	Falcon® Round-Bottom Polystyrene Tubes, 5 mL (Catalog #38007)
5 - 10 mL	Falcon® Round-Bottom Polystyrene Tubes, 14 mL (Catalog #38008) OR Falcon® Conical Tubes, 15 mL (Catalog #38009)

2. Add 1 part HetaSep™ to 5 parts whole blood. Mix well.
3. Centrifuge at 90 x *g* at room temperature (15 - 25°C) with the brake off, according to Table 2.

Table 2. Centrifuge Times Based on Sample Age

START VOLUME*	TUBE SIZE	CENTRIFUGE TIME (minutes)		
		Fresh blood	24-hr-old blood	48-hr-old blood
2 mL	5 mL	1	1	2
3 mL	5 mL	1	1	4
4 mL	5 mL	2	2	5
10 mL	14 mL	5	5	7

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

NOTE: If processing blood in a 50 mL tube, contact us at techsupport@stemcell.com for centrifuge speeds and times.

4. Remove sample from centrifuge and allow to sit undisturbed at room temperature for 10 minutes. This will allow further sedimentation of the RBCs 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 still remain in this fraction; this is expected.
6. Wash this fraction once with at least a 4-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 the brake off.

   NOTE: If excessive platelet contamination is expected, repeat this wash step.
7. Carefully remove supernatant 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 mL of medium).
8. Optional: Residual RBCs may be lysed with Ammonium Chloride Solution (Catalog #07800).

NOTE: The age of the blood sample affects 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. Hemolysis also makes the interface more difficult to see.

Related Products

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com/HSPCworkflow or contact us at techsupport@stemcell.com. For available fresh and cryopreserved peripheral blood, cord blood, and bone marrow products, visit www.stemcell.com/primarycells.

References

Regidor C et al. (1999) Umbilical cord blood banking for unrelated transplantation: evaluation of cell separation and storage methods. Exp Hematol 27(2): 380–5.

Rubinstein P et al. (1995) Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc Natl Acad Sci USA 92(22): 10119–22.