HetaSep™

For Isolating Human Nucleated Cells from Peripheral Blood

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 RBCs 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.

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.

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

Contains:

- Hetastarch (6% w/v)
- Sodium chloride
- Sodium lactate (anhydrous)
- Dextrose (hydrous)
- Calcium chloride dihydrate
- Potassium chloride
- Magnesium chloride hexahydrate

Handling / Directions for Use

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.

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

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

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. 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 no brake.
5. If excessive platelet contamination is expected, repeat this wash step.
6. 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).
7. Optional: Any residual RBCs may be lysed with Ammonium Chloride (Catalog #07800) if desired.

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.
2. Add 1 part HetaSep™ to 5 parts whole blood. Mix well.
3. Centrifuge sample at room temperature (15 - 25°C) at 90 x *g* 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 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 not have sedimented and thus 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 no brake.
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 - 1 mL of medium).
9. Optional: Any residual RBCs may be lysed with Ammonium Chloride (Catalog #07800), if desired.

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 the interface more difficult to see.

Table 1. Tube Size Recommendations for Centrifugation

WHOLE BLOOD VOLUME	RECOMMENDED TUBE SIZE
1 - 4 mL	Falcon™ 5 mL polystyrene round-bottom tubes (BD Catalog #352058)
5 - 10 mL	Falcon™ 14 mL round-bottom tubes (e.g. BD Catalog #352057) **OR** 15 mL conical tubes (e.g. Corning Catalog #430053)

Table 2. Centrifuge Times Based on Sample Age

START VOLUME*	TUBE SIZE	SPIN TIME (MIN)		
		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: Contact Technical Support for centrifuge speeds and times if processing blood in a 50 mL tube.

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 U S A 92(22): 10119–22.