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ProductInformation

HISTOPAQUE® 1083

Product Code **1083-1**
Storage Temperature: 2-8 °C

Product Description

In 1968, Boyum described methods for the isolation of mononuclear cells from circulating blood and bone marrow.[1] Boyum further stated that procedures used for the isolation of leukocytes from human peripheral blood are also applicable to blood from mice, rats and guinea pigs.[2] HISTOPAQUE 1083 has been designed to be used to separate blood from rats, mice and other mammals.[3-8]

In general, the procedures employed mixtures of polysaccharide and a radiopaque contrast medium. HISTOPAQUE 1083 is a solution of polysucrose and sodium diatrizoate, adjusted to a density of 1.083 g/ml. This medium facilitates rapid recovery of viable mononuclear cells from small volumes of blood.

Reagent

Polysucrose, 64 g/L and sodium diatrizoate, 100 g/L. Aseptically filtered.

Reagents and equipment required but not provided

- Isotonic Phosphate Buffered Saline or Cell Culture media, e.g.,
  RPMI 1640, Product Code R 6504
  Hanks' Balanced Salt, Product Codes H 2387, H 6648 or H 4641
  Dulbecco's PBS, Product Codes D 5652, D 8537, D 1408
- 15 ml or 50 ml centrifuge tubes
- Pipettes
- Centrifuge with swinging buckets capable of generating 400-1000xg force

Optional Equipment and Reagents

- ACCUSPIN TUBES™. Radiation sterilized, Product Codes A 1805 or A 2055. 12 or 50 ml capacity polypropylene tubes fitted with a high density polyethylene barrier, or frit. Used to keep blood from mixing with the HISTOPAQUE 1083 before centrifugation.
- ACCUSPIN TUBES. Non-sterile, Product Code A 1930. 50 ml capacity polypropylene tubes fitted with a high density polyethylene barrier , or frit. Used to keep blood from mixing with the HISTOPAQUE 1083 before centrifugation.
- Wright Stain, Modified (Manual), Product Code WS16
- Trypan Blue Solution, 0.4%, Product Code T 8154

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

This product is not intended for use with human blood.

Preparation Instructions

Ready to use. Warm to room temperature before use.

Storage/Stability

Store tightly closed at 2-8 °C. The expiration dating is printed on the bottle label. Do not freeze. If the product freezes, warm to room temperature and invert the bottle several times. If the solution is free of precipitate and clear, the product may be used. A cloudy appearance or precipitate indicates deterioration of the product. Protect from long term exposure to light. Short term exposure to light does not damage the solution.

Procedure

Anticoagulated blood is layered onto HISTOPAQUE 1083. During centrifugation, erythrocytes are aggregated by polysucrose and rapidly sediment; whereas, lymphocytes and other mononuclear cells remain at the plasma–HISTOPAQUE 1083 interface. Erythrocytes pellet to the bottom of the centrifuge tube. Due to the increased density of granulocytes, the granulocytes will not band with the mononuclear cells and will move towards the bottom of the centrifuge tube. Most extraneous platelets are removed by low speed centrifugation during the washing steps.

1. Add HISTOPAQUE 1083 to centrifuge tubes
   - Add 3.0 ml if using a 15 ml centrifuge tubes.
   - Add 15.0 ml if using 50 ml centrifuge tubes
2. Carefully layer whole blood onto the HISTOPAQUE 1083 surface
   - Layer 3.0 ml whole blood if using 15 ml centrifuge tubes
   - Layer 15.0 ml whole blood if using 50 ml centrifuge tubes
3. Centrifuge at 400xg for exactly 30 minutes at room temperature. Centrifugation at lower temperatures, such as 4 °C, may result in cell clumping or poor recovery. The brake on the centrifuge should be in the off position
4. After centrifugation, carefully aspirate, with a Pasteur or plastic pipet, the upper layer to within 2-3 mm of the opaque interface containing the mononuclear cells. Discard upper layer
5. Carefully transfer the opaque interface, containing the mononuclear cell band, with a Pasteur pipet, into a clean 15 ml or 50 ml conical centrifuge tube.
6. Add isotonic PBS or other appropriate cell culture medium (serum free) to the monuclear cells
   - Add 10 ml of if using 15 ml centrifuge tubes
   - Add 30 ml if using 50 ml centrifuge tubes
   - Mix tube by gentle inversion several times
7. Centrifuge at 250xg for 10 minutes. Aspirate the supernatant and discard.
8. Resuspend the cell pellet with 0.5 ml of isotonic PBS or appropriate cell culture medium, then
   - Add an additional 4.5 ml of isotonic PBS or cell culture medium if using 15 ml centrifuge tubes
   - Add an additional 10-15 ml of isotonic PBS or cell culture medium if using 50 ml centrifuge tubes
   - Mix several times by gentle inversion.
9. Centrifuge at 250xg for 10 minutes. Aspirate the supernatant and discard.
10. Repeat Steps 8-9 as needed. Typically 2 or 3 washes are needed to remove any remaining HISTOPAQUE 1083 from the mononuclear cells. After the final wash, resuspend the cells in isotonic PBS or cell culture medium
    - If using 15 ml centrifuge tubes, resuspend the pellet in 0.5 ml PBS or cell culture medium
    - If using 50 ml centrifuge tubes, resuspend the pellet in 1.5 ml PBS or cell culture medium
11. At this point a variety of assays can be performed. The procedures are chosen according to individual discretion.

Troubleshooting Guide

1. The blood used for separation should be fresh and free of clots. Collect venous blood in preservative-free anticoagulant. For best results the blood should be processed as soon as possible. Delays in processing the blood can result in lower cell recoveries or loss of viability. EDTA and heparin are commonly used anticoagulants. EDTA should be used in a range of 1.25 to 1.75 mg/ml. Heparin should be used in a range to 15 to 30 units/ml. Recoveries from heparin treated blood will drop noticeably after 2 hours. Recoveries from EDTA treated blood will drop after 2 hours, and noticeably after 6 hours.
2. The purity of the cell population obtained by this procedure may be determined by automation or by performing a Romanowsky stain (i.e., Wright Stain) on a cytospin slide prepared from the cells collected in Step 10 of the Procedure. Slides prepared from air dried cell suspensions will show a significant drying artifact. Cytospin preparations will show better cell morphology and are highly recommended.
3. Viability may be determined by use of trypan blue exclusion. If cell viability is <80%, replacement of PBS with a cell culture medium such as RPMI 1640 fortified with 5% fetal bovine serum may prove helpful.
4. Prediluted blood may be used with this procedure. Blood may be diluted with isotonic PBS or appropriate cell culture medium. Recommended ratio for dilution is 1 part blood to 1 part diluent. Bone marrows should be diluted 1:2 or 1:4, depending upon the cell count.
5. Removing excess amounts of HISTOPAQUE 1083 with the mononuclear cell band increases granulocyte contamination.
6. Removing excess amounts of plasma with the mononuclear cell band may promote contamination with plasma proteins or platelets.
7. To remove contaminating platelets, a second centrifugation can be performed. The mononuclear cell band may be layered over a 8.125% sucrose solution prepared in deionized water. Layer the mononuclear cells onto the sucrose gradient and centrifuge at 300xg for 10-15 minutes. Platelets should not penetrate the sucrose gradient and the mononuclear cells should pass through. The sucrose gradient may also be placed over the HISTOPAQUE 1083 to band the mononuclear cells at the sucrose/Histopaque interface.
8. Protocol modifications may be necessary if working with animals other than rats or mice.

References

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7. Feldman, D.L. and Mogelesky, T.C., Use of Histopaque for isolating mononuclear cells from rabbit blood., J. Immunol. Methods, **102**, 243-49 (1987).
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