

TECHNICAL BULLETIN

Red Blood Cell Clearance Protocol for STEMvision™

Introduction

STEMvision™ is an automated instrument and computer system designed specifically for imaging and scoring hematopoietic colonies in the colony-forming cell (CFC) assay. In order for the STEMvision™ software to accurately count and identify colonies, red blood cell (RBC) background in the dish must be minimized. A hematocrit of less than 1% in the dishes to be counted is recommended (see Figures 1 and 2).

STEMCELL Technologies Inc. has developed a protocol for removing RBCs using HetaSep™. This protocol has been designed to minimize time required for RBC removal for ease of incorporation into an institution's workflow. This RBC removal protocol can also be used if hematopoietic colonies will be scored manually.

FIGURE 1. Acceptable background devoid of RBCs (note right inset image)

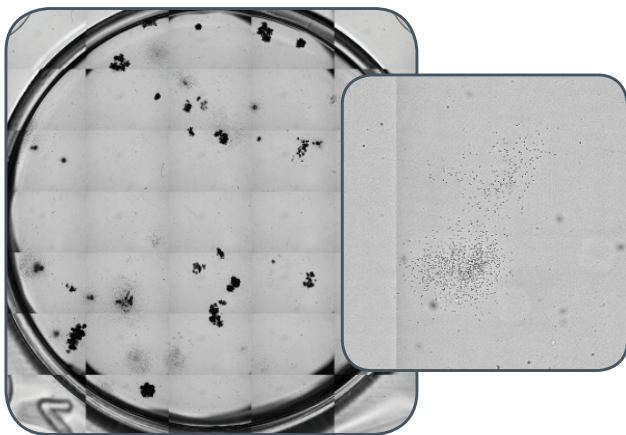
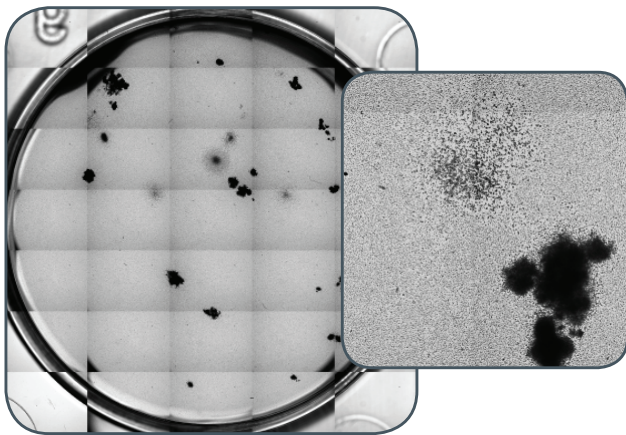


FIGURE 2. Unacceptable background with RBCs (note right inset image)



Materials

- Microcentrifuge tubes (0.5 mL)
- Dulbecco's Phosphate Buffered Saline (D-PBS; Catalog #37350)
- HetaSep™ (Catalog #07806/07906)

Protocol

1. Perform any cell counting methods completed as part of standard sample analysis prior to proceeding with the red cell clearance protocol. Determine the sample volume required as per standard protocols.
2. In a 0.5 mL microcentrifuge tube, mix cord blood sample (50 μ L or less) with the appropriate volume of D-PBS to give a final volume of 200 μ L. Add 40 μ L of HetaSep™ and mix by pipetting. Multiple samples may be processed at one time for increased workflow efficiency. Note: A starting sample volume of 50 μ L effects a sample dilution factor of 0.208.
3. Incubate at 37°C for 20 minutes. Following the incubation, take care when handling the tubes, so as not to disturb the layers. Do not allow more than 30 minutes to pass from the start of the incubation before proceeding to the next step.
If more than 30 minutes has passed, mix the layers by pipetting and repeat Step 3 to ensure optimal cell recovery.
4. Transfer as much of the top layer as possible to a clean tube without disturbing the red cell pellet and gently mix using a pipette to obtain an even cell suspension. A volume of 180 μ L or more should be recovered but will depend on the sample hematocrit and the starting sample volume.
5. Divide the required sample volume determined in Step 1 by the dilution factor to determine the volume of post-processed sample required for inoculation in MethoCult™. For example, if a sample volume of 15 μ L was calculated from the initial cell count in Step 1, the tube of MethoCult™ would be inoculated with 72.1 μ L (15 μ L \div 0.208) of the post-processed sample.
6. Proceed with the remainder of the CFC assay set-up according to standard protocols.

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The number of CFCs is not affected by the HetaSep™ procedure. The number of total nucleated cells (TNCs) may be slightly lower. This may be due to the fact that some cells are missed during the transfer step, particularly if the individual performing the procedure is attempting to not disturb the red cell pellet. The loss of these cells does not affect the number of CFCs.

Recovery from HetaSep™ Procedure

	START COUNT		POST-PROCESS COUNT	
	Average	Standard Deviation	Average	Standard Deviation
TNC	87%	14%	82%	14%
CFC	100%	19%	113%	34%

Cord blood samples (n=23) were processed using the HetaSep™ protocol outlined above. Each sample was processed twice simultaneously. One set was counted after processing, prior to plating in MethoCult™ ("post-process count"), and one set was plated in MethoCult™ based on pre-processing counts ("start count"). TNCs were counted after processing in both sets, even though this was not used to determine plating in MethoCult™ in the "start count" set. This was done to show that the numbers of TNCs did not differ between groups.