

TITLE: Modified ISHAGE CD34+ Enumeration Assay CD34/V1

PRINCIPLES OF THE PROCEDURE:

To stain a sample for the presence of hematopoietic stem cells, which are CD34+. The results obtained from this assay are reported to the Stem Cell Laboratory and will be used to determine the appropriate treatment of the patient. This includes, but is not limited to; determining if/when a patient will undergo pheresis or what dose of a particular drug said patient will be administered.

SPECIMEN REQUIREMENTS:

Whole blood or Apheresis product from the Bone Marrow Procurement Lab.

REAGENTS:

TOPRO-3 IODIDE, Stock Conc. 1mM: Molecular Probes, Catalog # T-3605
PE-IgG1: Caltag Laboratories, Catalog # MG104
FITC-CD45 BD Biosciences, Catalog #340664
PE-CD34 BD Biosciences, Catalog #340669
STEM CELL CONTROL (STEM-TROL) Beckman Coulter, Catalog # IM3632
Ammonium Chloride lysing solution, made fresh daily, refer to procedure in this manual for details on its preparation.
SPHERO ACCUCOUNT FLUORESCENT PARTICLES 5.23um Spherotech, Catalog # ACFP-50-5
HANKS BALANCED SALT SOLUTION, without Phenol Red, Roswell Park Media Facility

EQUIPMENT & INSTRUMENTATION

Vortex
Pipettes
Variable speed pipette
Serological pipettes
Timer
12 by 75 plastic test tubes
FACSCanto

PROCEDURE:

V1:

ADD 100ul OF PATIENT SAMPLE TO 30ul OF V1 ANTIBODY IN "V1" TUBE
(LYSE WITH OTHER TUBES AFTER INCUBATION *see CD34 directions below).
(do **NOT** add topro or beads to V1 tube!)

CD34:

1. COUNT SAMPLE
2. DILUTE SAMPLE (WITH HANKS SOLUTION) TRY TO GET A COUNT CLOSE TO **30,000**
3. LABEL TUBE #1-#5
4. ADD PATIENT NAME TO TUBE **#1 - #3** (*tubes #4 and #5 are HD*)
5. PIPETTE 20ul OF **CD45/34** INTO TUBES **#1, #2 AND #4**
6. PIPETTE 20ul OF **CD45/CTRL** ANTIBODY TO TUBES **#3 AND #5**
7. PIPETTE 20ul OF **STEM CONTROL (SC)** INTO TUBES **#4 AND #5**
8. PIPETTE 100ul OF **PATIENT SAMPLE (DILUTED ONE)** INTO TUBES **#1-#3 VORTEX**
9. PIPETTE 100ul OF HEALTHY **DONOR** INTO TUBES **#4 AND #5 VORTEX**
10. PIPETTE 20ul OF **TOPRO** INTO ALL TUBES (**#1-#5**)
VORTEX
11. INCUBATE FOR **20 MINUTES** AT ROOM TEMP. IN DARK
12. ADD **2ml** OF **LYSE** TO EACH TUBE(**#1-#5**)
VORTEX
13. INCUBATE FOR **10 MINUTES** AT ROOM TEMP IN DARK
14. PIPETTE **100ul** OF **BEADS** INTO EACH TUBE (**#1-#5**)
15. RUN ON CANTO
16. ANALYZE DATA

PROCEDURAL NOTES:

- Thoroughly mix the fluorospheres and Stem-Trol Control cells at the beginning of the experimental set up and directly before use. Avoid the formation of air bubbles in the fluorospheres.
- All volume transfer manipulations should involve the 'reverse pipetting' technique. Use the same pipettes throughout the execution of this procedure.
- Ensure that the samples are run within 1 hour of adding the fluorospheres.
- Confirm that the WBC count is not greater than 30×10^6 cells/ml. Dilute the cells with HBSS to about 15×10^6 if necessary. See dilutions sheet.
- Record the dilution factor.

QUALITY CONTROL GUIDELINES:

NORMAL HEALTHY DONOR is run in parallel.

EXPECTED VALUES:

None determined

REPORTING RESULTS & CALCULATIONS

The absolute number of CD34 is calculated as: (Clean FS vs SC (R7) / Singlet Beads (R9)) X Bead Count on vial X any Dilution factor X Normalization.
The normalization factor is equal to 1 for patients and 5 for our Stem-trols.
This formula is in the excel worksheet.

REFERENCES:

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