Method for HemaFlow™

Step 1	Remove HemaFlow™ Reagent from the -20ºC freezer and thaw at room temperature. Mix the contents gently so that no bubbles are created.
Step 2	Prepare MNC target cells and adjust the concentration to 50,000 cells/mL. For purified cells, adjust to between 5,000 and 10,000 cells/mL
Step 3	Dispense 0.09mL of the ready-to-use HemaFlow™ Reagent into each well.
Step 4	Dispense 0.01mL of the cell suspension so that the total volume is 0.1mL and the final cell concentration is reduced 10 fold.
Step 5	Mix the contents of each well 2-3 times with the pipette, without forming bubbles. DO NOT use a vortex mixer.
Step 5	Replace the lid and transfer the 96-well plate to a humidified chamber and transfer the chamber to a humidified incubator at 37ºC with 5% CO_2 and, if possible 5% O_2.
Step 6	Culture human cells for 7-9 days, mouse cells for 5 days.
Step 7	**For flow cytometers with multi-well capability:** (a) Centrifuge the cells directly in the plate (b) Remove and discard supernatant (c) Add required dilution of CD or other antibodies and, if possible counting beads, mix and incubate for 20 mins. or according to the manufacturer's instructions. (d) Perform flow cytometric acquisition for each individual well. If counting beads are not available, ensure that the flow cytometer can reliably perform a cell count. (e) If required, verify results using a HALO® Research assay for same population.
Step 7a	**For flow cytometers with tube capability:** (a) Remove cells from individual replicate wells and transfer to a tube. Alternatively, the contents of replicate wells can be pooled into the same tube. (b) Centrifuge the cells and remove and discard supernatant. (c) Add required dilution of CD or other antibodies and, if possible counting beads. Mix and incubate for 20 mins or according to the manufacturer's instructions. (d) Perform flow cytometric acquisition. If counting beads are not available, ensure that the flow cytometer can reliably perform a cell count. (e) If required, verify results using a HALO® Research assay for the same population.
Step 8	Analyze the flow cytometric data using normal gating procedures.

Tips

1. Always ensure that pipettes (manual and electronic) are professionally calibrated to maintain accuracy and avoid disensing errors.
2. Always include a background control (cells in HemoGro™ Medium) or any other controls that will prove the assay is working correctly.