



Tutorial

| Isolation of Target Cells From
Bone Marrow

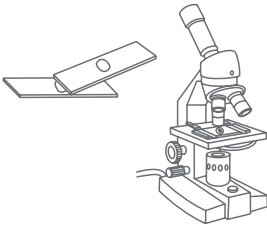


This tutorial is provided solely as a procedure workflow example. Solutions, quantities, containers, and equipment should be adjusted or replaced to suit the needs of your application(s) and each specimen processed.

IMPORTANT

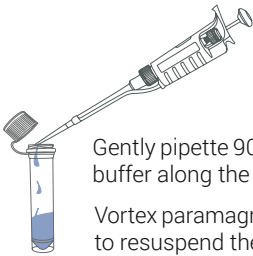
Before proceeding with the following Steps, remove reagents from the refrigerator to ensure that the blocking buffer, used to reduce non-specific binding and retain cell morphology, and paramagnetic antibodies are at room temperature.

Step 1 (Optional)

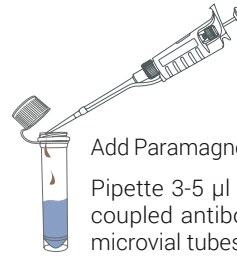


Prior to target cell enrichment, prepare a slide smear from the bone marrow specimen for morphological review. The slide smear will serve as a reference that sufficient viable cells are present. Stain appropriately to identify the presence of target cells on your smear.

Step 2



Gently pipette 900-950 μ l of a WaveSense blocking buffer along the inside edge of a 2 mL microvial. Vortex paramagnetic antibodies at a reduced speed to resuspend the paramagnetic beads.



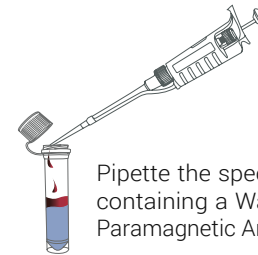
Add Paramagnetic Antibodies (typically 3 - 5 μ l). Pipette 3-5 μ l of the appropriate paramagnetic coupled antibody to the specimen in the 2 mL microvial tubes.

Step 3

Transfer specimen to the 2 mL microvial.

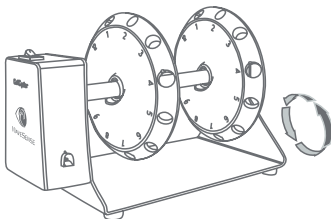


Aliquot 50-100 μ l of specimen from original container.



Pipette the specimen into the 2 mL microvial containing a WaveSense blocking buffer and Paramagnetic Antibodies.

Step 4

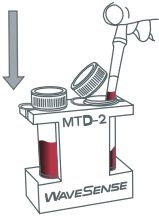


Cap and gently invert each 2 mL microvial several times (3-4). Place all 2 mL microvials on the CellCycler™ (#A2102-1) for 15-30 minutes.

The CellCycler is used to keep cells and reagents in uniform suspension during the incubation of cells with Paramagnetic Antibodies.

Step 5

At the end of the incubation period, loosen the cap of each 2 mL microvial and place it firmly into a MTD-2 (#A4101-5) magnetic tube dock for 5 minutes.



At the end of the 5 minute incubation, remove supernatant from the side opposite the magnet of the MTD-2.

After supernatant is removed with an aspirator or transfer pipette, place the supernatant into a waste container labeled biohazard.



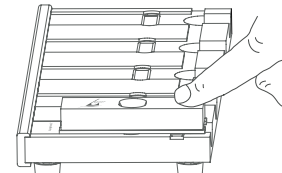
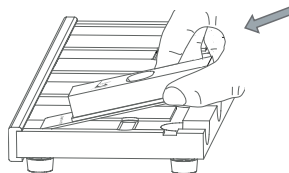
Resuspend target cells in 1 mL of a WaveSense blocking buffer. Pipette the buffer down the side where the bead line appears.

Cap vials and place back into the CellCycler for 2 minutes to mix.

NOTE: Supernatant contains the remaining cells that are not CD138+ captured and may be used for further analysis.

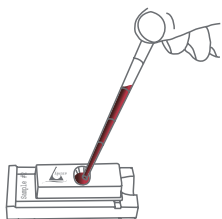
Step 6

Label each EpiSep HS with a pencil and insert into the MSD5 (#A1104-1) five position magnetic slide dock. Make sure it clicks to indicate full insertion.



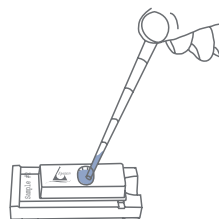
Step 7

Add the 1 mL of suspension from each microvial to the inner wall of the EpiSep HS. Be careful not to create bubbles. Allow 1 minute for the suspension to drain.

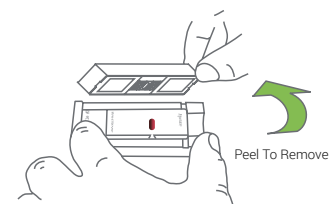


Rinse all 4 walls of the well with 4 drops of a WaveSense blocking buffer. Next, rinse all 4 walls with 4 drops of absolute ethanol or Carnoy's. Allow the slide to drain for 1 minute.

NOTE: Use only absolute ethanol if creating a slide for PlasmaCell^{QC} Stain. If FISH-only use Carnoy's fixative.



Remove the EpiSep HS cap by gently pulling up while slide is still wet and in the MSD5. Place it into a humidity-controlled environment for 5 minutes. (45-50% humidity and 27°C)

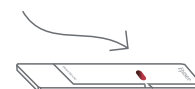


Step 8

Remove slide from the humidity chamber when condensation appears on the back of the slide.



Inspect the monolayered target area by viewing slide under a phase contrast microscope. A 12 millimeter coverslip can be used to cover the target area.





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The WaveSense products listed in this tutorial are intended for In Vitro Diagnostic Use. Refer to Product Specification Sheets for complete information on individual products. EpiSep is a registered trademark of WaveSense Incorporated.

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