Uniform Specimen Process

Tutorial | Isolation of Target Cells From Bone Marrow

WAVESENSE

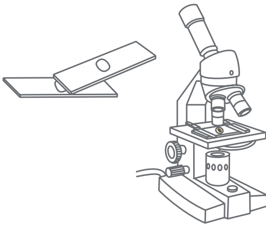
Enriching Patient Lives

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.

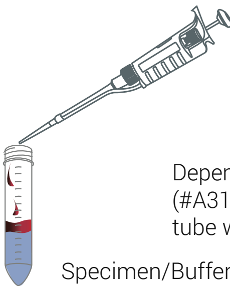
Step 1

(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



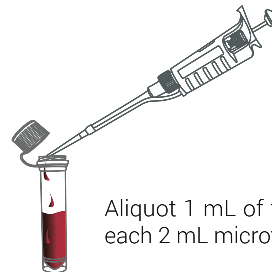
Create a homogeneous mixture by combining a WaveSense blocking buffer with the amount of bone marrow received (0.5-1.0 mL).

Depending on the number of EpiSep® HS (#A3104-10) needed, fill the 15 mL conical tube with 4-9 mLs of blocking buffer.

Specimen/Buffer volume to EpiSep HS conversion chart:

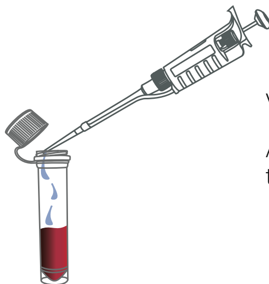
4 mLs = 4 EpiSep HS
5 mLs = 5 EpiSep HS
6 mLs = 6 EpiSep HS
7 mLs = 7 EpiSep HS
8 mLs = 8 EpiSep HS
9 mLs = 9 EpiSep HS

Mix well by slow inversion of the tube.



Aliquot 1 mL of the homogeneous mixture into each 2 mL microvial.

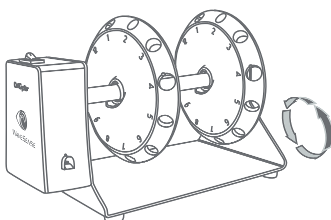
Step 3



Vortex paramagnetic antibodies to resuspend the paramagnetic beads.

Add 7-20 µls of the appropriate Paramagnetic Antibody to the specimen located in the 2 mL microvial tubes.

Step 4



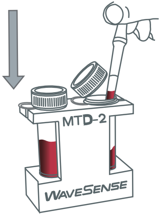
Cap and gently invert each 2 mL microvial several times (3-4). Place all 2 mL microvials on the CellCycler™ (#A2102-1) for 15-30 minutes.

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

Note: lower quantities of paramagnetic coupled antibody may require longer mixing time on the CellCycler.

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.

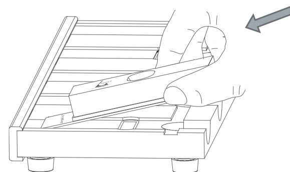
After supernatant is removed with an aspirator or transfer pipette, place the supernatant into a waste container labeled biohazard.

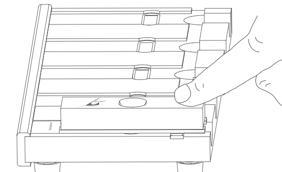
NOTE: Supernatant contains the remaining cells that are not CD138+ captured and may be used for further analysis.

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.

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.



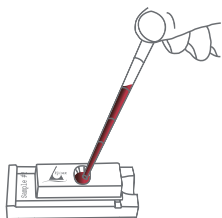


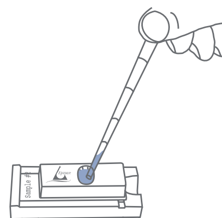
Step 7

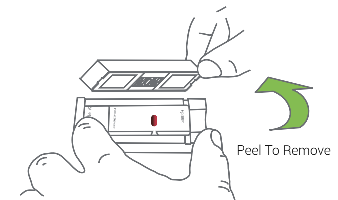
Add the 1 mL of suspension from each microvial to the inner wall of the EpiSep HS. Be careful not to create bubbles. Allow 1 minute for the suspension to drain.

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)

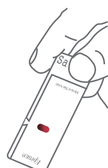




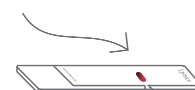


Step 8

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.



Tutorial | General Procedure for the Isolation of Target Cells from Bone Marrow

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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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