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

Uniform Specimen Process - Single-Tube Method

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 media, 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.

Use an appropriate cellular stain to identify the presence of target cells on your smear.

Step 2

Using a 15 mL conical tube, create a homogeneous mixture by combining RPMI 1640 media with the bone marrow specimen.

- Depending on plasma cell percentage, allocate 100-200 µL of bone marrow for each EpiSep® HS (<3%=200 µL, ≥3%=100 µL).
- As specified in the chart below, use RPMI 1640 media to bring the specimen/media mixture volume to a ratio of 1 mL for each EpiSep HS.

Specimen/media volume to EpiSep HS conversion chart:

1 mL = 1 EpiSep HS	6 mL = 6 EpiSep HS
2 mL = 2 EpiSep HS	7 mL = 7 EpiSep HS
3 mL = 3 EpiSep HS	8 mL = 8 EpiSep HS
4 mL = 4 EpiSep HS	9 mL = 9 EpiSep HS
5 mL = 5 EpiSep HS	10 mL = 10 EpiSep HS

Step 3

- Vortex to resuspend the paramagnetic antibodies.
- As specified in the chart below, add 10 µL of the appropriate paramagnetic antibody to the specimen/media mixture in the 15 mL tube for each EpiSep HS.

Paramagnetic antibody volume to EpiSep HS conversion chart:

10 µL = 1 EpiSep HS	60 µL = 6 EpiSep HS
20 µL = 2 EpiSep HS	70 µL = 7 EpiSep HS
30 µL = 3 EpiSep HS	80 µL = 8 EpiSep HS
40 µL = 4 EpiSep HS	90 µL = 9 EpiSep HS
50 µL = 5 EpiSep HS	100 µL = 10 EpiSep HS

Step 4

Cap and gently invert the 15 mL conical tube several times (3-4). Attach the 15 mL CellCycler Tube Adapter to the 15 mL conical tube then insert it into the CellCycler. Set voltage to 7.5V DC (14.0 - 16.0 RPM) 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 the 15 mL conical tube and place it firmly into a MTD-15 (#A4102-1) magnetic tube dock for 5 minutes. ***NOTE:*** *Do not incubate more than 5 minutes in the MTD-15 as it may induce aggregation.*
- At the end of the 5-minute incubation, remove supernatant from the side opposite the MTD-15 magnet. Be careful not to scrape cells off the side of the conical tube.
- After the supernatant is removed with an aspirator or transfer pipette, place the supernatant into a waste container labeled biohazard. ***NOTE:*** *Supernatant contains non-target cells that may be useful for further analysis.*
- As specified below, resuspend target cells with 5-10 mL of hypotonic solution (0.075M KCl). Cap the 15 mL conical tube, invert to mix cells and place in a 37°C environment for 10 minutes.

  Specimen volumes < 500 µL = 5 mL of 0.075M KCl.
  Specimen volumes ≥ 500 µL = 10 mL of 0.075M KCl.

Step 6

- At the end of the 10-minute incubation period, remove the 15 mL conical tube from the incubator. Slowly add 1 mL of Carnoy's fixative. Invert gently to mix.
- Loosen the cap of the 15 mL conical tube and place it firmly into a MTD-15 (#A4102-1) magnetic tube dock for 5 minutes. ***NOTE:*** *Do not incubate more than 5 minutes in the MTD-15 as it may induce aggregation.*
- At the end of the 5-minute incubation, remove supernatant from the side opposite the MTD-15 magnet. Be careful not to scrape cells off the side of the conical tube.
- Resuspend the cells with Carnoy's fixative using the same volume conversion in Step #2. ***Note:*** *For storage of the tube for reflex testing, please refer to www.wavesense.com/faq*

Step 7

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 8

- Add 1 mL of suspension from the 15 mL conical tube to the inner wall of each EpiSep HS. Allow 1 minute for the suspension to drain or until all fluid has drained from the well. ***NOTE:*** *Do not let the slides sit and dry with the EpiSep HS cap still attached.*
- Remove EpiSep HS cap by gently pulling up while slide is still wet and in the MSD5. Place slides into a humidity-controlled environment for 5 minutes. (45-50% humidity)

Step 9

- Slides are now ready for your laboratory's standard FISH protocol or special stain process. Inspect monolayered cell target area by viewing slides under a phase contrast microscope.

15339 Barranca Pkwy., Irvine, CA 92618
800.807.7760
info@wavesense.com
wavesense.com

WAVESENSE
Enriching Patient Lives