Technical Data Sheet

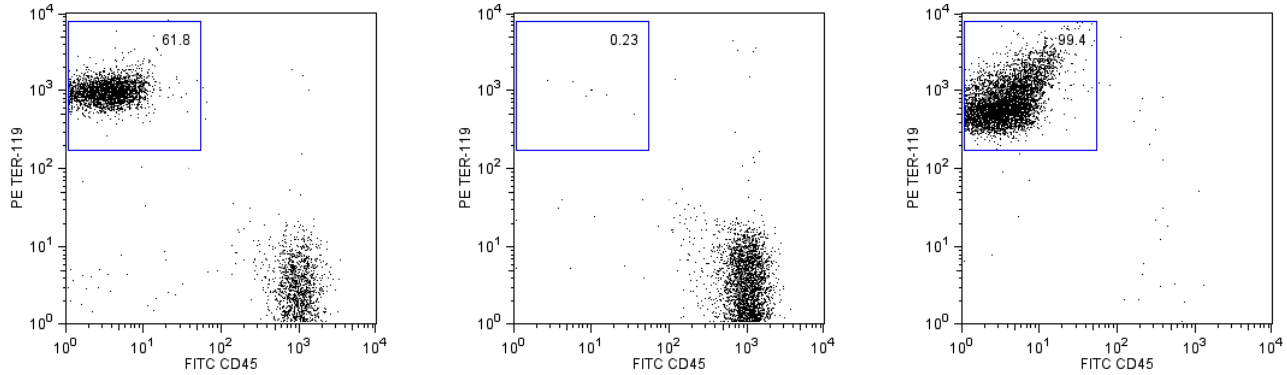
Anti-Mouse Ter-119 Particles - DM

Product Information

Material Number:	**558536**
Size:	5.0 ml
Clone:	TER-119
Immunogen:	Mouse Fetal Liver
Isotype:	Rat (WI) IgG2b, κ
Reactivity:	QC Testing: Mouse
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

The TER-119 antibody specifically binds to a 52 kDa molecule associated with glycophorin A on cells of the erythroid lineage in embryonic yolk sac, fetal liver, newborn liver, adult bone marrow, adult peripheral blood, and adult lymphoid organs. The TER-119 antigen is expressed on erythroid cells from pro-erythroblast through mature erythrocyte stages, but not on cells with BFU-E or CFU-E activities. The TER-119 epitope is not detected on hematopoietic stem cells, lymphoid cells, myeloid cells, or erythroleukemia lines. The TER-119 mAb is a component of the "lineage cocktail" used in studies of hematopoietic progenitors to detect, or deplete cells committed to the hematopoietic lineages.



Depletion of mouse TER-119-positive cells from spleen cells. *Splenocytes were labeled with BD IMag™ anti-mouseTER-119 particles- DM as described in the protocol. After labeling, the cells were separated using the BD IMagnet™, and the negative (TER-119-) and positive (TER-119+) fractions were collected. Please refer to the Separation Flow Chart to identify the separated cell populations represented in this figure. For flow cytometric analysis, fresh spleen cells (left panel), the depleted fraction (center panel), and the positive fraction (right panel) were stained with PE anti-mouse TER-119 mAb TER-119 (Cat. No. 553673) and FITC anti-mouse CD45 mAb 30-F11 (Cat. No.553080). The percent TER-119+ cells in each sample is given.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Store undiluted at 4°C.

Application Notes

Application

Cell separation	Routinely Tested

Recommended Assay Procedure:

Mouse spleen cell or bone marrow suspension is labeled with BD IMag™ anti-mouse TER119 Particles - DM according to the following Protocol. This labeled cell suspension is then placed within the magnetic field of the BD IMagnet (Cat. no. 552311). Labeled cells migrate toward the magnet (positive fraction), leaving the unlabeled cells in suspension so they can be collected (depleted fraction). The tube is then removed from the magnetic field and additional buffer is added for resuspension of the positive fraction. The magnetic separation steps are diagrammed in the Separation Flow Chart. Both depleted and positive fractions can be evaluated in downstream applications such as flow cytometry.

MAGNETIC LABELING AND DEPLETION PROTOCOL

1. Prepare 1X BD IMag™ buffer: Dilute BD IMag™ Buffer (10X) (Cat. no. 552362) 1:10 with sterile distilled water or prepare Phosphate Buffered Saline (PBS) supplemented with 0.5% BSA, 2 mM EDTA, and 0.1% sodium azide.

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2. Prepare a single-cell suspension from the lymphoid tissue of interest according to standard laboratory procedures. Remove clumps of cells and/or debris by passing the suspension through a 70-µm nylon cell strainer. Count the cells.
3. Wash cells with an excess volume of 1X BD IMag™ buffer, spin and carefully aspirate all the supernatant.
4. Vortex the BD IMag™ anti-mouse TER-119 Particles - DM thoroughly, and add 50 µl of particles for every 10^7 total cells.
5. MIX THOROUGHLY. Incubate in the refrigerator (8°C-11°C and not on ice) for 30 minutes exactly.*
6. Wash the labeled cells with a 10X excess volume of 1X BD IMag™ buffer, centrifuge at 300 x g for 7 minutes, and carefully aspirate ALL the supernatant.
7. Bring the labeling volume up to 20 to 80 x 10^6 cells/ml with 1X BD IMag™ buffer.
8. Transfer the labeled cells to a 12 x 75 mm round-bottom test tube (eg, BD Falcon™, Cat. no. 352058), maximum volume added not to exceed 1.0 ml. Place this positive-fraction tube on the BD IMagnet™ (horizontal position) for 6 to 8 minutes.*

    For greater volume, transfer the cells to a 17 x 100 mm round-bottom test tube (eg, BD Falcon™, Cat. no. 352057), maximum volume added not to exceed 3.0 ml. Place this positive-fraction tube on the BD IMagnet™ (vertical position) for 8 minutes.*

9. With the tube on the BD IMagnet™ and using a sterile glass Pasteur pipette, carefully aspirate the supernatant (depleted fraction) and place in a new sterile tube.
10. Remove the positive-fraction tube from the BD IMagnet™, and add 1X BD IMag™ buffer to the same volume as in Step 7. Resuspend the positive fraction well by pipetting up and down 10 to 15 times, and place the tube back on the BD IMagnet™ for 6 to 8 minutes.*

    For 17 x 100 mm tube: Place on the BD IMagnet™ for 8 minutes.*

11. Using a new sterile Pasteur pipette, carefully aspirate the supernatant and combine with the enriched fraction from Step 9 above.
12. Repeat Steps 10 and 11. The combined depleted fraction contains cells with no bound antibodies or magnetic particles.
13. To increase the purity of the combined depleted fraction, place the tube containing the combined enriched fraction on the BD IMagnet™ for another 6 minutes.*

    For 17 x 100 mm tube: Place on the BD IMagnet™ for 10 minutes.*

14. Carefully aspirate the supernatant and place in a new sterile tube. This is the twice-depleted fraction. The cells are ready to be processed for downstream applications.
15. The positive-fraction cells remaining in the original tube can be resuspended in an appropriate buffer or culture medium for downstream applications, including flow cytometry, if desired.
16. Samples of the total cell suspension and the positive and depleted fractions should be analyzed by flow cytometry to evaluate the efficiency of the cell-separation procedure.

NOTES:

Hints for successful cell preparation:

After the final wash, resuspend the cells at a relatively high concentration in 1X BD IMag™ buffer and proceed to step 3.

It is highly recommended to do step 13 to ensure a higher rate of depletion.

* Avoid nonspecific labeling by working quickly and adhering to recommended incubation times.

The concentration of BD IMag™ anti-mouse TER-119 Particles - DM suggested in the protocol has been optimized for the purification of TER-119-positive erythoid lineage cells from spleen cells. When labeling target cell populations present at lower frequencies, fewer BD IMag™ particles can be used. Conversely, when labeling target cell populations that are present at higher frequencies, more particles should be used. To determine the optimal concentration of the BD IMag™ anti-mouse TER-119 Particles - DM for a particular application, a titration in two-fold increments is recommended.

Suggested Companion Products

Catalog Number	Name	Size	Clone
552311	Cell Separation Magnet	each	(none)
552362	Buffer (10X)	100 ml	(none)
553673	PE Rat Anti-Mouse TER-119/Erythroid Cells	0.2 mg	TER-119

Product Notices

1. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. BD IMag™ particles are prepared from carboxy-functionalized magnetic particles which are manufactured by Skold Technology and are licensed under US patent number 7,169,618.

DEPLETION FLOW CHART

(The circled numbers correspond to the steps of the Protocol on the following page.)