EpCAM-Magnetic Beads

Product description

Positive CTC Selection Assay (EpCAM-magnetic beads) is designed to provide simple, sensitive and quantitative assessment of performing CTC enrichments by magnetically labeled CTC from peripheral blood. The EpCAM-magnetic beads use microscale particles with a magnetic core and couple with antibodies that recognize the EpCAM antigen. By using the magnetic field and via the antibody specific binding ability, the magnetically labeled EpCAM cells could be temporarily immobilized on the column wall, so the depleted EpCAM cells in the supernatant can be discard easily and efficiently under magnetic attraction. Captured magnetically labeled EpCAM cells are visible and respond quickly to magnets that are easy for manipulation.

Properties

This product is for research use only.

Components	2mL EpCAM-magnetic beads, human: magnetic beads conjugated to monoclonal antibody (isotype: mouse IgG2b).
Capacity	For ~10^9 total cells, up to 20 separations.
Product format	EpCAM-magnetic beads are supplied in stabilizing buffer and 0.01% sodium azide.
Storage	Store protected from light at 2−8 °C. Do not freeze. The validity is warranted for 6 months.

Applications

Detection and enrichment of disseminated carcinoma cells in peripheral venous blood, for example, of patients with epithelial cancers, for subsequent analysis, e.g., flow, immunofluorescence, enumeration, cultivation, or RT-PCR.

Protocol

Preparation of whole blood sample

1. Collect blood sample into two tubes: discard the first 3-5 ml of blood drawn in the first tube to avoid skin epithelial contamination then collect up to 7.0 mL of venous blood sample into the second one with anticoagulant EDTA and kept in room temperature. Whole blood sample should be processed within 24 hours of collection.
2. For each sample to be processed, coat a 2mL tube with 1mL of binding buffer and rotate at 4°C until use.
3. Prepare the 50mL Leucosep (with frit) tube by adding 15mL of Ficoll® Paque PLUS and centrifuging the tube at 1000xg for 60 seconds.
4. Gently add 5mL of phosphate buffered saline (PBS, pH=7.4) to the Leucosep tube when ready to process the blood sample.
5. Immediately add blood sample to Leucosep tube by first decanting it from the blood collection tube into the Leucosep tube. Then, gently wash down the walls of the blood collection tube twice, each time with 10mL of PBS.
6. Immediately centrifuge the tubes at 800xg for 15 minutes with the brake setting to OFF.
7. Decant about 20mL of the supernatant in Leucosep tube into a new 50mL conical tube. Gently swirl the remaining supernatant to dislodge any cells that may be stuck to the wall of the Leucosep tube and then decant it into the same conical tube. Rinse the wall of the Leucosep tube with 5mL of PBS and add that to the same 50mL conical tube. Be careful not to suction the Ficoll® Paque PLUS through the frit; avoid pressing the pipette against the frit.
8. Centrifuge the 50mL conical tube from Step 7 at 500 x g for 10 minutes, followed by using a serological pipette to gently aspirate off the supernatant as much as possible without disturbing the pellet.
9. Use a 10mL pipette to remove the remaining supernatant closer to the bottom of the tube and avoid disturbing the pellet (up to ~500µl buffer may be left remaining). Keep the pellet on ice. Decanting the supernatant is NOT recommended because the pellets might be very loose in clinical samples.
10. Gently tap the tube to loosen pellet and tap patiently until the pellet is completely resuspended. Gently resuspend the cells with P1000 pipette. If necessary, add up to 300 µL of binding buffer to the tube.
11. Remove and discard the binding buffer from the tube prepared in Step 2. Transfer cell suspension into the 2mL tube. Rinse the residual cells in the 50mL conical tube with binding buffer and transfer to the same 2mL tube. The final volume should be approximately 1mL. Keep cell suspension on ice until use.
12. Proceed to magnetic labeling.

Magnetic labeling

▲Using pre-cooled buffers and slightly vortex the beads before use, to make sure there are no clumps left, in order to prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲Using positive and negative controls to verify the beads labeling. Whole blood sample spiked with tumor cells as positive control, whole blood sample from a healthy donor as negative control.

▲Volumes for magnetic labeling of 100µl/test are for up to 5×10^7 total cells. When working with fewer than 5×10^6 cells, use the same volumes as indicated. Scale up the reagent volume when working with higher cell numbers.

13. Add 40ul of Fc blocker into isolated PBMC and keep the tube at room temperature for 5 minutes.
14. Add 100 µL of EpCAM-magnetic beads per 5×10^7 to the 2mL tube from Step 11.
15. Mix well and incubate for 2 hours in the refrigerator (2−8 °C) on the slowly rotating mixer.

Magnetic separation with the magnetic separation rack

16. After cell incubation, place the 2mL eppendorf tube on the

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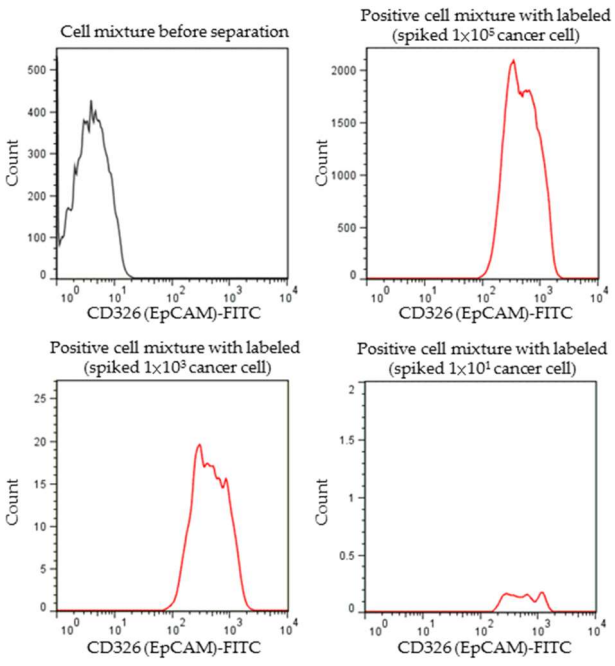
magnetic separation rack for 60 seconds to immobilize the beads at tube wall.

17. Discard the supernatant by aspiration with a pipette.
18. Remove the tube from the magnetic separation rack.
19. Add 1 mL binding buffer and re-suspend the beads by slightly vortex in order to wash the cells.
20. Place the 2mL tube on the magnetic stand for 60 seconds to immobilize the beads at tube wall.
21. Discard the supernatant, and then remove the tube from the magnetic separation rack.
22. Collect the magnetic beads-labeled cells by adding 200ul binding buffer for further experiments and analysis.

Example of a separation using EpCAM-magnetic beads

Tumor cells enrichment from whole blood sample spiked with cells from a colon cancer cell line (HCT116) using EpCAM-magnetic beads.

Cells are fluorescently stained with CD326 (EpCAM)-FITC and analyzed by flow cytometry



Trouble Shooting

1. EpCAM-magnetic beads binding is low:
   - Make sure the magnetic beads are sufficiently suspended before use.
   - Mix magnetic beads and sample thoroughly and continuously with a tilt rotation device.
   - Increase the amount of magnetic beads used for capture.
2. Non-specific and background binding is high:
   - Reduce the usage amount of magnetic beads per test according to the binding capacity.
3. Magnetically labeled EpCAM cells do not collect on the magnet:
   - Aspirate slowly and remove the supernatant without disturbing the bead ring / pellet to avoid bead loss.
   - Make sure the tube is in directly contact with the magnetic separation rack.

Contact Information

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References

1. Went, P.T., Lugli, A., Meier, S., Bundi, M., Mirlacher, M., Sauter, G., and Dirnhofer, S. (2004). Frequent EpCAM protein expression in human carcinomas. Hum Pathol 35, 122-128.
2. Xu, H., Aguilar, Z.P., Yang, L., Kuang, M., Duan, H., Xiong, Y., Wei, H., and Wang, A. (2011). Antibody conjugated magnetic iron oxide nanoparticles for cancer cell separation in fresh whole blood. Biomaterials 32, 9758-9765.
3. Zborowski, M., and Chalmers, J.J. (2011). Rare cell separation and analysis by magnetic sorting. Anal Chem 83, 8050-8056.
4. Imrich, S., Hachmeister, M., and Gires, O. (2012). EpCAM and its potential role in tumor-initiating cells. Cell Adh Migr 6, 30-38.
5. Grover, P.K., Cummins, A.G., Price, T.J., Roberts-Thomson, I.C., and Hardingham, J.E. (2014). Circulating tumour cells: the evolving concept and the inadequacy of their enrichment by EpCAM-based methodology for basic and clinical cancer research. Ann Oncol 25, 1506-1516.
6. Plouffe, B.D., Murthy, S.K., and Lewis, L.H. (2015). Fundamentals and application of magnetic particles in cell isolation and enrichment: a review. Rep Prog Phys 78, 016601.
7. Raimondi, C., Nicolazzo, C., and Gradilone, A. (2015). Circulating tumor cells isolation: the "post-EpCAM era". Chin J Cancer Res 27, 461-470.
8. Schneck, H., Gierke, B., Uppenkamp, F., Behrens, B., Niederacher, D., Stoecklein, N.H., Templin, M.F., Pawlak, M., Fehm, T., Neubauer, H., et al. (2015). EpCAM-Independent Enrichment of Circulating Tumor Cells in Metastatic Breast Cancer. PLoS One 10, e0144535.

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