Magnetic bead–based workflows

Isolation of circulating tumor cells using Dynabeads magnetic beads

Introduction

Circulating tumor cells (CTCs) are gaining importance as prognostic markers for early detection of disease and for monitoring of treatment response. Because of the low number of CTCs in circulation, highly sensitive methods are necessary to capture and detect them down to the single cell level.

Invitrogen™ Dynabeads™ magnetic beads provide an automation-friendly tool for isolation of circulating biomarkers using either a positive or negative isolation approach. Positive isolation enables the capture of CTCs by targeting cancer-specific markers, whereas negative isolation can deplete leukocytes from blood samples for marker-independent CTC enrichment, leaving the target cells untouched. This application note evaluates the feasibility of Dynabeads magnetic beads in both positive and negative isolation workflows for CTC research, highlighting their adaptability and performance in addressing this challenging yet promising area of cancer research.

Methods

Positive isolation of CTCs was evaluated by utilizing Dynabeads magnetic beads coupled to monoclonal antibodies (mAbs) targeting epithelial cell adhesion molecule (EpCAM). Contrived samples were prepared at a titration of 1, 3, 5, 7, and 10 epithelial cancer cells spiked into 7.5 mL of whole blood diluted 1:2 in Gibco™ Dulbecco's Phosphate-Buffered Saline (DPBS) using a micromanipulator to create five independent samples of varying cell concentrations. **Invitrogen™ Dynabeads™ Epithelial Enrich** (M-450) magnetic beads* were used to capture CTCs from whole blood samples. The captured cells were then washed and lysed directly on the Dynabeads magnetic beads using a magnetic stand. For control samples, single cells were lysed directly. The lysates were mixed with Invitrogen™ Dynabeads™ Oligo(dT)$_{25}$ beads for mRNA isolation and downstream RT-qPCR analysis. The cells were quantified using Applied Biosystems™ TaqMan™ Assay chemistry targeting cytokeratin 19 (CK19).

Additionally, we compared the performance of Invitrogen™ Dynabeads™ MyOne™ Epoxy magnetic beads* (coupled to anti-EpCAM mAbs) and Invitrogen™ Dynabeads™ MyOne™ Streptavidin C1 and T1 beads (coated with biotinylated anti-EpCAM mAbs) to the performance of Dynabeads Epithelial Enrich magnetic beads. This comparison was performed at a concentration of 5 cells/sample from 4 different donors. RT-qPCR with TaqMan Assays was used to analyze the expression of CK19 and CD45 from CTCs and white blood cells, respectively, as a measure of cell capture efficiency and background.

While Dynabeads Epithelial Enrich magnetic beads are highly recommended for efficient capture of epithelial cells, Dynabeads MyOne Streptavidin T1 beads are suggested for capturing other cell types using target-specific antibodies.

Figure 1 details the general workflow proposed for positive isolation to capture epithelial cells from whole blood samples.

* M-450 beads are 4.5 µm. MyOne beads are 1 µm.

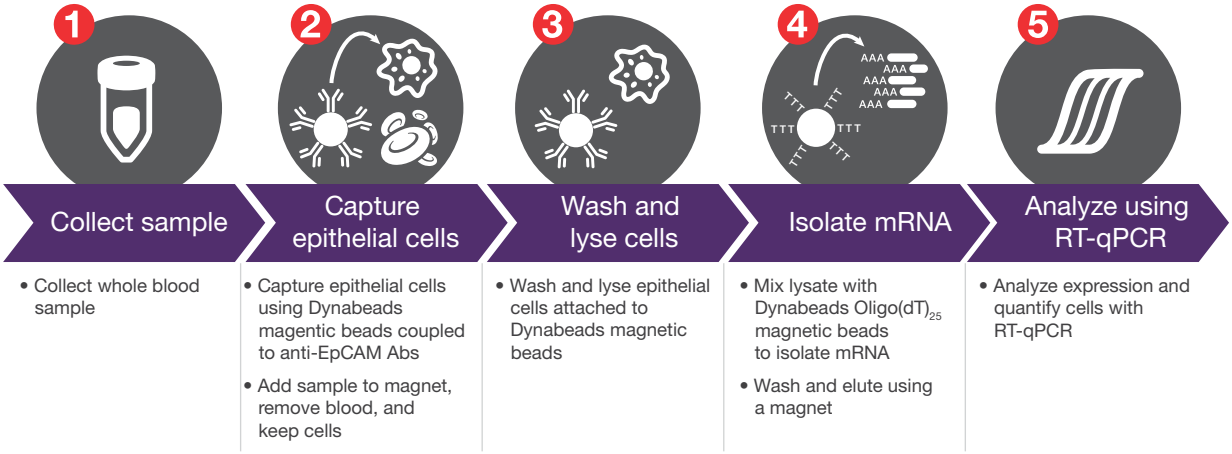

Figure 1. Positive isolation workflow for detection of CTCs using Dynabeads magnetic beads coupled to anti-EpCAM Abs for epithelial cell capture. Following capture, magnetic beads can be washed using a magnetic stand, and subsequent lysate can be utilized for mRNA isolation to quantify cells with expression markers using RT-qPCR.

Negative isolation of CTCs was evaluated by depleting CD45-positive leukocytes collected from blood-derived samples using Dynabeads magnetic beads coupled to anti-CD45 mAbs. This approach allows for CTCs to remain unbound to beads as they are free of this surface marker. Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coat and incubated with **Invitrogen™ Dynabeads™ MyOne™ CD45 Leukocyte Depletion** beads. The sample was removed from the leukocytes bound to the beads using a magnetic stand. After magnetic separation, the cells remaining in the supernatant were analyzed by flow cytometry to determine percent depletion. Dynabeads MyOne CD45 Leukocyte Depletion beads were compared to Invitrogen™ Dynabeads™ CD45 beads as well as to Dynabeads MyOne Streptavidin C1 and T1 beads coated with the same anti-CD45 antibody. Figure 2 details the general workflow for negative isolation to capture and remove leukocytes from the PBMC sample.

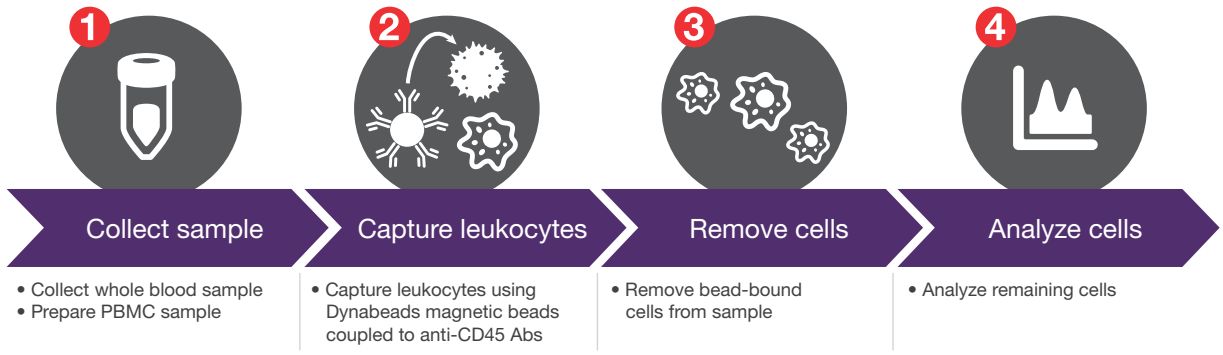

Figure 2. Negative isolation workflow for the capture and removal of leukocytes bound to Dynabeads magnetic beads coupled to anti-CD45 Abs. In this study, flow cytometry was utilized to evaluate bead performance in negative isolation workflows.

Results and discussion

The results for the positive isolation workflow show the C_t values for CK19 expression from 1:2 diluted whole blood samples containing 1, 3, 5, 7, or 10 cells were within the range determined by the controls. This indicates a highly sensitive capture down to one single cell (Figure 3).

Real-time PCR results were comparable across several Dynabeads platforms, indicating similar efficiency in cell capture (Figure 4A). However, the amount of nonspecific binding of leukocytes was lower for Dynabeads MyOne Streptavidin C1 and T1 beads (~10x lower; $\Delta C_t = 3.4$), compared to Dynabeads Epithelial Enrich beads (Figure 4B).

Flow cytometry confirmed cell count reduction post-depletion using Dynabeads MyOne CD45 Leukocyte Depletion beads or Dynabeads CD45 beads. However, beads of different size exhibited different binding properties (Figure 5).

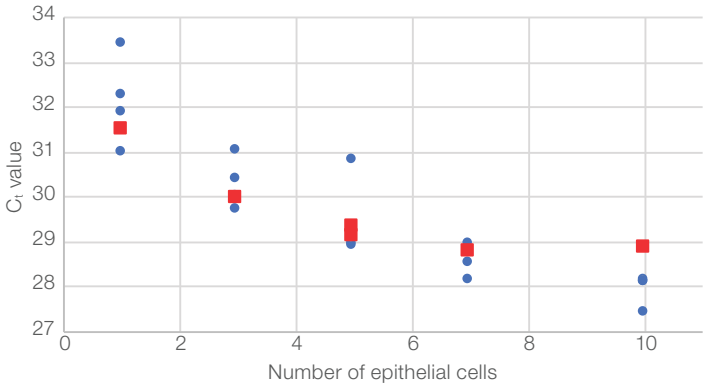

Figure 3. C_t values obtained for different number of cells. Single cells (1, 3, 5, 7, and 10 cells) were spiked into and captured from 7.5 mL of 1:2-diluted whole blood (red) or lysed directly (controls; blue).

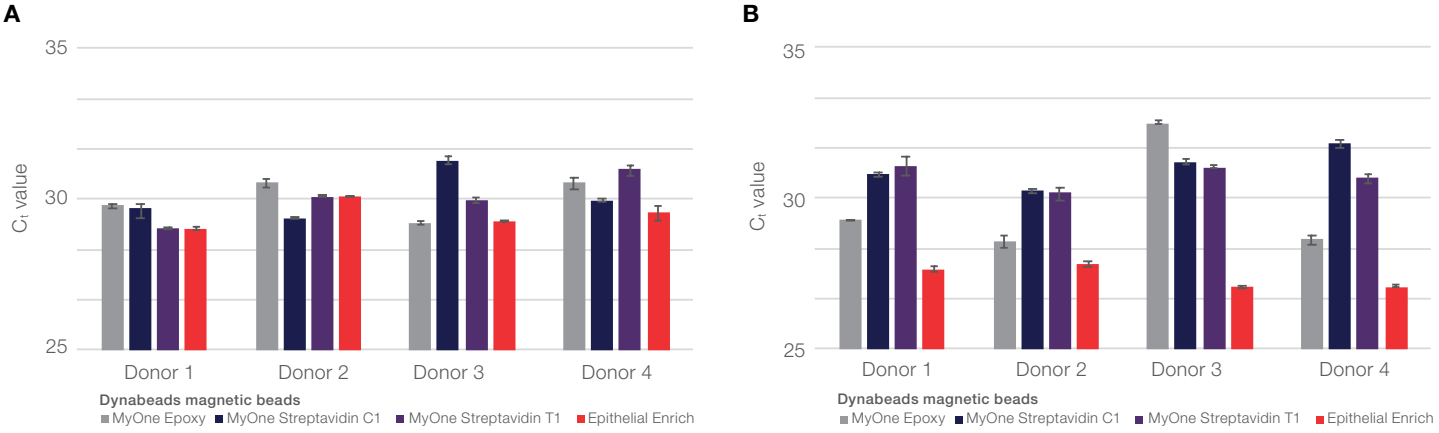

Figure 4. Comparison of RT-qPCR results from positive CTC isolation workflows. Five cells were spiked into blood from four donors and captured using four types of Dynabeads magnetic beads coupled to anti-EpCAM Abs. **(A)** CK19 and **(B)** CD45 expression levels were analyzed.

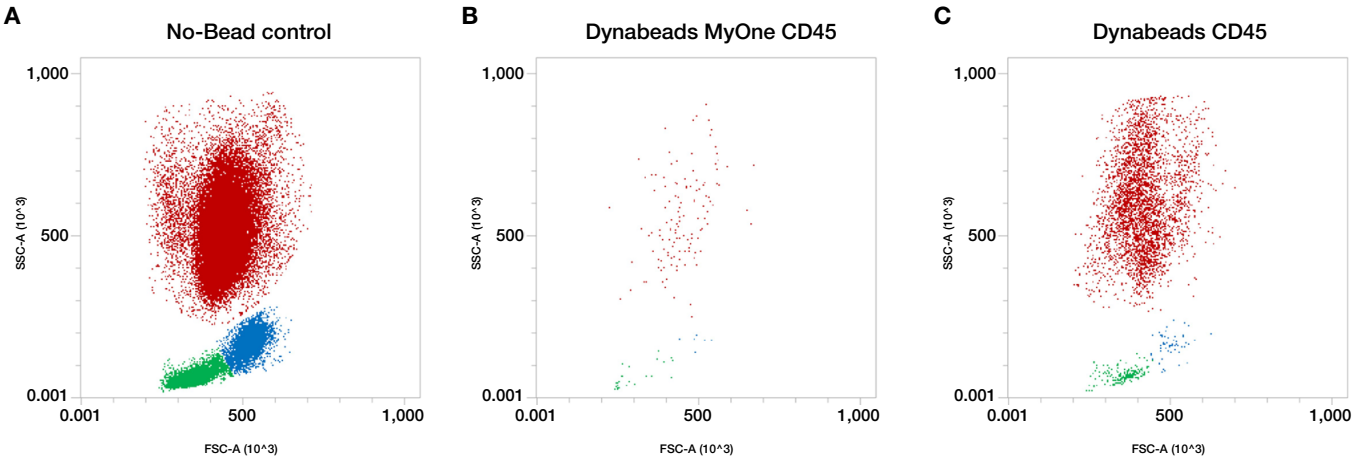

Figure 5. Scatter plots for leukocytes showing lymphocyte (green), monocyte (blue), and granulocyte (red) depletion. The figure shows leukocytes **(A)** before depletion and after depletion using **(B)** Dynabeads MyOne CD45 Leukocyte Deletion beads or **(C)** Dynabeads CD45 beads.

Figure 6 shows a comparison of depletion between Dynabeads CD45 beads and Dynabeads MyOne CD45 Leukocyte Depletion beads. While both bead types showed similar depletion efficiency in PBMCs, Dynabeads MyOne CD45 Leukocyte Depletion beads showed higher depletion in lysed blood samples.

Small beads have been shown to be beneficial for the depletion of cells with low expression of surface markers. Dynabeads CD45 magnetic beads depleted only ~88% of CD45-low granulocytes (Figure 7). In contrast, applying Dynabeads MyOne CD45 Leukocyte Depletion beads also efficiently depleted CD45-low granulocytes.

Dynabeads Streptavidin T1 beads coated with anti-CD45 antibody showed similar cell depletion efficiency as Dynabeads MyOne CD45 beads while Dynabeads Streptavidin C1 beads revealed slightly lower cell depletion (Figure 8).

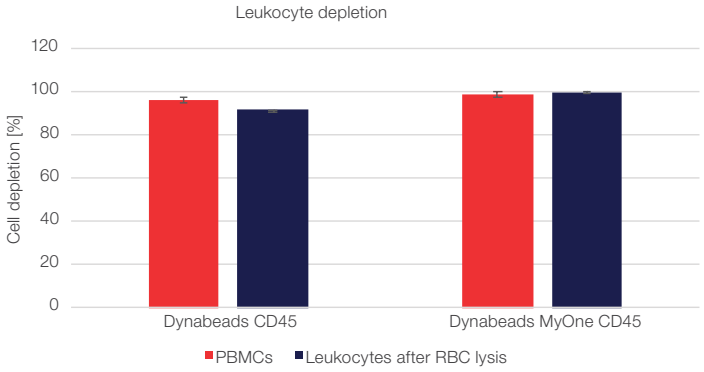

Figure 6. Leukocyte depletion from PBMCs and from lysed red blood cells (RBCs) using Dynabeads CD45 and Dynabeads MyOne CD45 Leukocyte Depletion beads determined by flow cytometry.

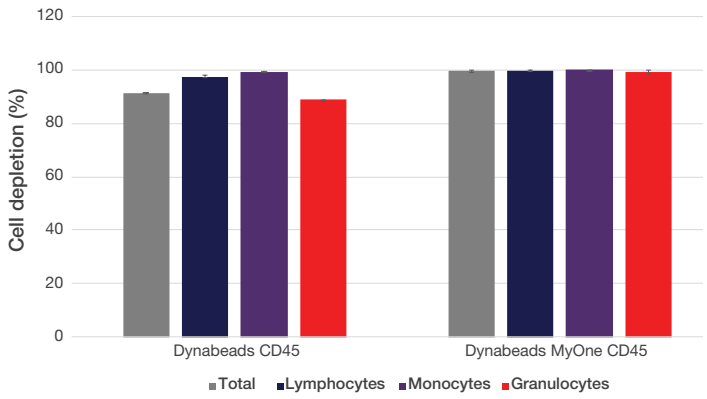

Figure 7. White blood cell depletion efficiency after RBC lysis of Dynabeads CD45 and Dynabeads MyOne CD45 Leukocyte Depletion beads.

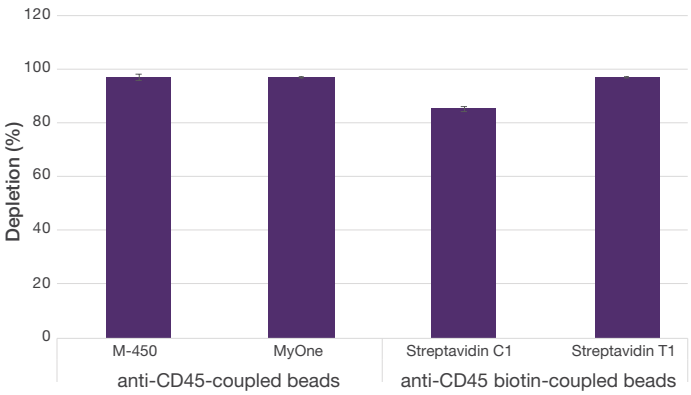

Figure 8. Comparison of cell depletion efficiency comparing covalently coupled Dynabeads (M-450 and MyOne) magnetic beads, and Dynabeads MyOne Streptavidin beads coated with the same anti-CD45 antibody. C1 = carboxylic acid–activated; T1: tosyl-activated.

Conclusions

Dynabeads magnetic beads can be coupled to antibodies targeting cancer-specific markers, such as EpCAM, for direct cell capture, or to anti-CD45 mAbs for leukocyte depletion for CTC enrichment, demonstrating feasibility for both positive and negative isolation of CTCs. RT-qPCR results after epithelial cell capture using Dynabeads magnetic beads showed capture sensitivity down to single cells. Flow cytometry results after leukocyte depletion using Dynabeads magnetic beads revealed Dynabeads MyOne CD45 Leukocyte Depletion magnetic beads are more efficient at capturing cells with low expression of surface markers (e.g., granulocytes expressing low CD45) compared to Dynabeads CD45 magnetic beads. However, the depletion efficiency is similar when PBMCs are used as the starting material.

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Ordering information

Product	Cat. No.
Dynabeads Epithelial Enrich	16103D
Dynabeads MyOne CD45 Leukocyte Depletion	11170D, 11171D
Dynabeads CD45	11153D
Dynabeads MyOne Streptavidin C1	65001
Dynabeads MyOne Streptavidin T1	65601
DynaMag-2 Magnet	12321D
DynaMag-15 Magnet	12301D
Attune Flow Cytometer	Various available
Phosphate-Buffered Saline (DPBS, 10X), Dulbecco's formula	14190

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