Magnetic bead–based workflows

Isolation of circulating tumor cells using Dynabeads magnetic beads

Introduction

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

Invitrogen™ Dynabeads™ magnetic beads provide an automation-friendly tool for isolation of circulating biomarkers. Generally, large beads are optimal for working on open platforms, while smaller beads are optimal for microfluidics. Positive isolation can be utilized to separate CTCs expressing cancer-specific markers, whereas negative isolation can be utilized to deplete leukocytes from blood samples for marker-independent CTC enrichment, leaving the target cells untouched. Here we evaluate Dynabeads magnetic beads for feasibility in both positive and negative CTC isolation workflows.

Methods

Positive isolation of CTCs was evaluated by utilizing Dynabeads magnetic beads coupled to monoclonal antibodies (Abs) 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 Thermo Scientific™ Phosphate-Buffered Saline (DPBS), Dulbecco's formula, 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). Furthermore, Invitrogen™ Dynabeads™ MyOne™ Epoxy magnetic beads* coupled to anti-EpCAM Abs and Invitrogen™ Dynabeads™ MyOne™ Streptavidin C1 and T1 beads coated with biotinylated anti-EpCAM Abs were compared to Dynabeads Epithelial Enrich magnetic beads at a concentration of 5 cells/sample from four different donors. RT-qPCR with TaqMan Assays was used to analyze expression of CK19 and CD45 from CTCs and white blood cells, respectively, as a measure of cell capture. 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.

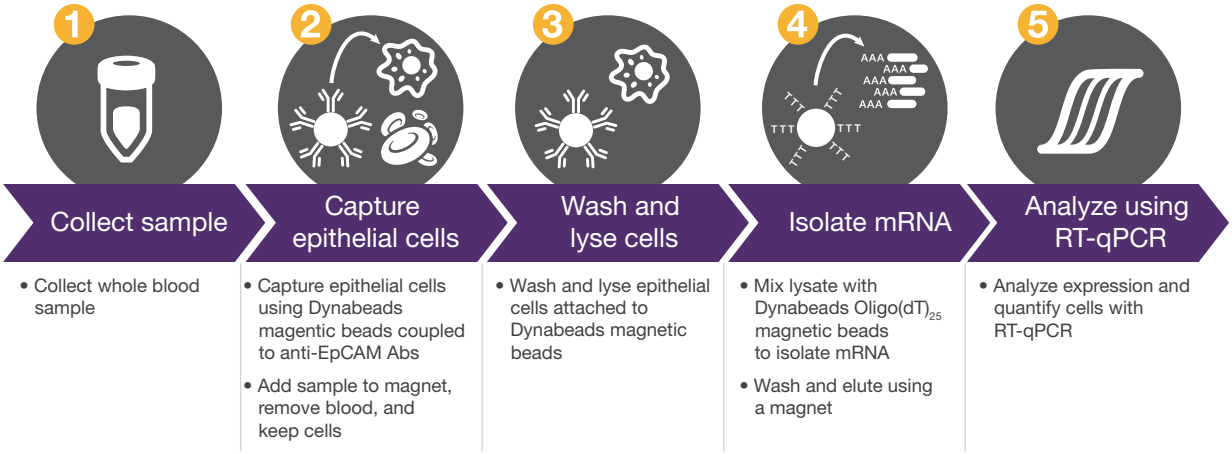

Figure 1. Positive isolation workflow for detection of CTCs using Dynabeads magnetic beads coupled to anti-EpCAM Abs for epithelial cell capture. Following capture, magnetic beads can be washed using a magnetic stand, and subsequent lysate can be utilized for mRNA isolation to quantify cells with expression 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 Abs, allowing for CTCs to remain unbound to beads as they are free of this surface marker. Mononuclear cells (MNCs) were isolated from buffy coat and incubated with Invitrogen™ Dynabeads™ CD45 beads (M-450) for leukocyte depletion. The sample containing CTCs was removed from the leukocytes bound to the beads using a magnetic stand. After magnetic separation, the cells remaining in the supernatant were stained with PE-labeled anti-CD45 and analyzed by flow cytometry for percent depletion. Dynabeads CD45 beads were compared to different surface-activated MyOne beads as well as to streptavidin beads coated with the same anti-CD45 antibody. Figure 2 details the general workflow proposed for negative isolation to capture and remove leukocytes using Dynabeads CD45 beads.

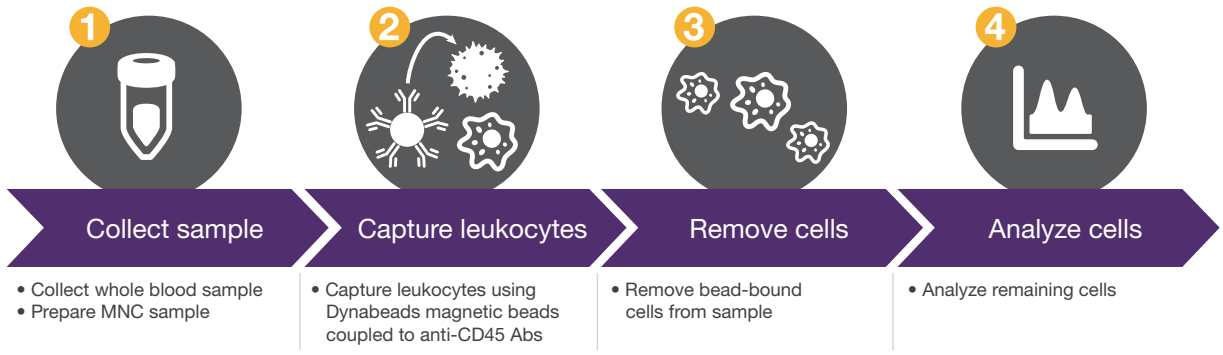

Figure 2. Negative isolation workflow for the capture and removal of leukocytes bound to Dynabeads CD45 beads. In this study, flow cytometry was utilized to evaluate bead performance in negative isolation workflows.

Results and discussion

The C_t values for CK19 expression from cells captured using the positive isolation workflow described above were within the range determined by the controls, indicating highly sensitive capture down to one single cell when comparing 1:2-diluted whole blood samples containing 1, 3, 5, 7, or 10 cells to the control samples (Figure 3).

Real-time PCR results were comparable across all 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 histograms confirmed cell count reduction post-depletion using Dynabeads CD45 beads (Figure 5). Figure 6 shows a comparison of depletion between Dynabeads CD45 beads and Dynabeads MyOne Epoxy beads coupled to anti-CD45 Abs. A higher number of beads per cell required for Dynabeads MyOne beads is due to the smaller bead size compared to that of Dynabeads CD45 beads. However, note the total bead mass was ~10x lower with the Dynabeads MyOne magnetic beads. Dynabeads MyOne beads with different surface activations and the corresponding streptavidin beads coated with the same anti-CD45 antibody revealed different depletion efficiencies (Figure 7).

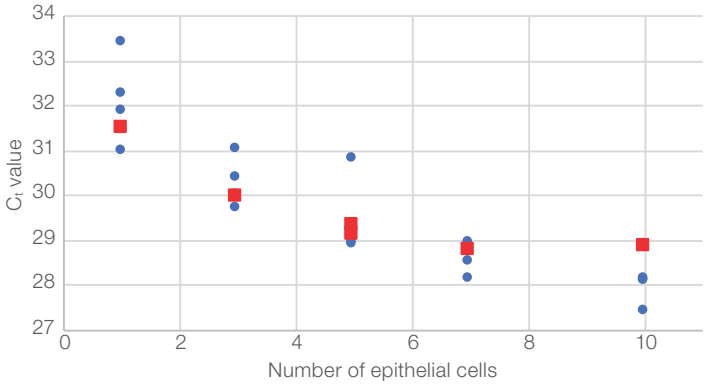

Figure 3. C_t values obtained for different number of cells. Single cells (1, 3, 5, 7, and 10 cells) were spiked into and captured from 7.5 mL of 1:2-diluted whole blood (red) or lysed directly (controls; blue).

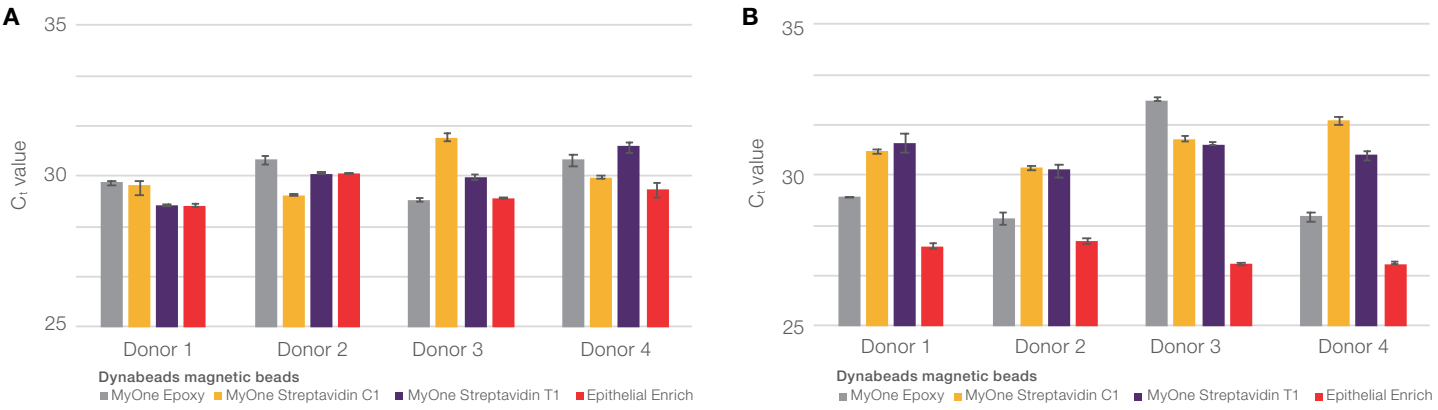

Figure 4. Comparison of RT-qPCR results from positive CTC isolation workflows. Five cells were spiked into blood from four donors and captured using four types of Dynabeads magnetic beads coupled to anti-EpCAM Abs. **(A)** CK19 and **(B)** CD45 expression levels were analyzed.

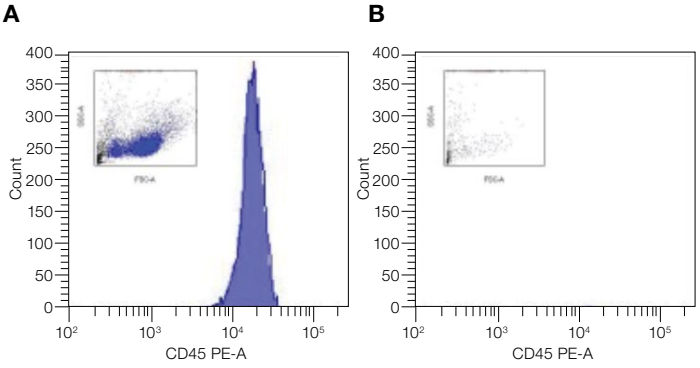

Figure 5. Fluorescence histograms for CD45-positive MNCs and scatter plots analyzed using flow cytometry (A) before and (B) after depletion using Dynabeads CD45 magnetic beads.

Epoxy-activated beads showed the highest depletion efficiency while carboxyl acid–activated beads demonstrated the least efficiency. However, different MyOne Streptavidin beads coated with anti-CD45 Abs showed only minor differences.

Different size beads exhibited different binding properties. 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 ~80% of CD45-low granulocytes (Figure 8). Cell depletion improved when Dynabeads CD15 magnetic beads were added, which directly targeted granulocytes. In contrast, applying anti-CD45 coupled Dynabeads MyOne beads achieved similar depletion efficiency as that of the combination of Dynabeads CD45 and CD15 magnetic beads.

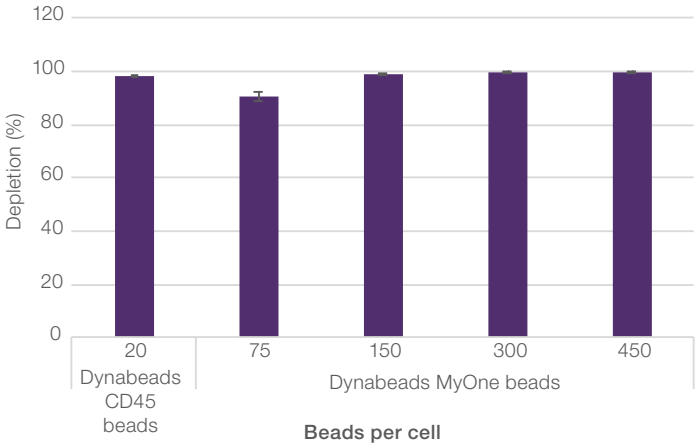

Figure 6. Cell depletion efficiency of Dynabeads CD45 beads, and Dynabeads MyOne Epoxy beads coupled to anti-CD45 Abs, determined by flow cytometry.

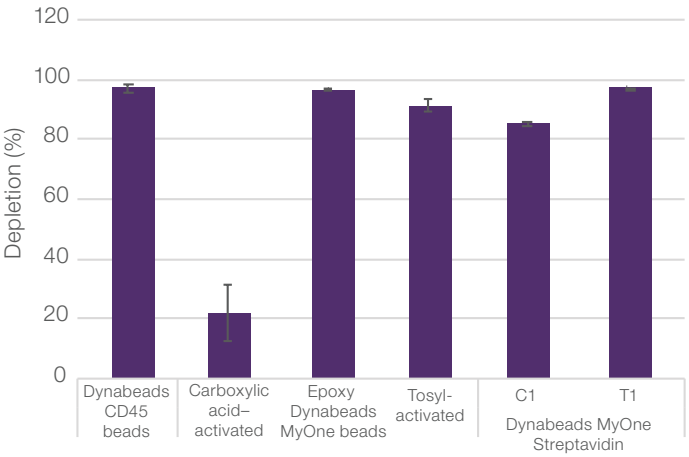

Figure 7. Comparison of cell depletion efficiency using different surface-activated Dynabeads MyOne beads, Dynabeads CD45 beads, and MyOne Streptavidin beads. C1 = carboxylic acid–activated; T1: tosyl-activated.

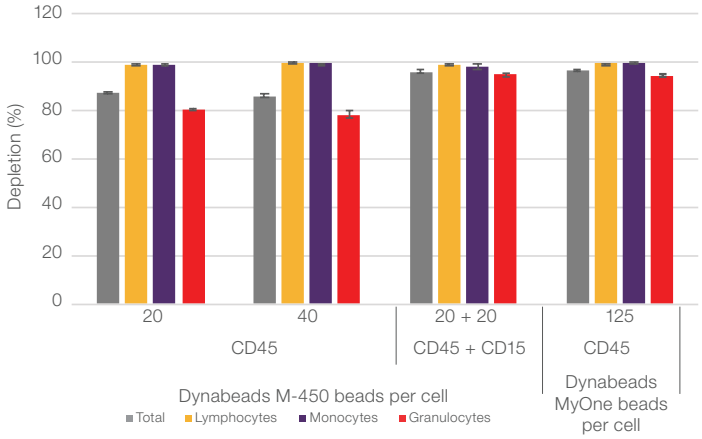

Figure 8. White blood cell depletion efficiency after RBC lysis of Dynabeads CD45 (M-450), CD15 (M-450) beads, and MyOne Epoxy beads coupled with anti-CD45 Abs.

Conclusions

The findings obtained from this study revealed that Dynabeads magnetic beads can isolate CTCs with high specificity and sensitivity for both positive and negative isolation techniques. Dynabeads magnetic beads can be coupled to antibodies targeting cancer-specific markers, such as EpCAM, for direct cell capture, or to anti-CD45 Abs for leukocyte depletion for CTC enrichment, demonstrating feasibility for both positive and negative isolations. Flow cytometry results using negative isolation workflows and Dynabeads magnetic beads revealed Dynabeads MyOne 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, this is not relevant when MNCs are used as the starting material. Although Dynabeads MyOne magnetic beads do require a higher number of beads, less total bead mass is required to achieve efficient cell capture or depletion in comparison to that of 4.5 µm magnetic beads.

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Ordering information

Product	Cat. No.
Dynabeads Epithelial Enrich	16102
Dynabeads CD45	11153D
Dynabeads CD15	11137D
Dynabeads MyOne Streptavidin C1	65001
Dynabeads MyOne Streptavidin T1	65601
Dynabeads MyOne Epoxy, for OEM and industrial use only	34001D
DynaMag-2 Magnet	12321D
DynaMag-15 Magnet	12301D
Attune Flow Cytometer	Various available
Phosphate-Buffered Saline (DPBS, 10X), Dulbecco's formula	J61917-K3

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