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# Dynabeads® Epithelial Enrich

**Catalog no. 16102** **Store at 2˚C to 8˚C**

**Rev. Date:** March 2012 (Rev. 006)

## Product Contents

| Product contents | Volume |
|---|---|
| Dynabeads® Epithelial Enrich | 5 mL |

**Product capacity**
Whole blood: 200 mL
MNC: ~2 × $10^9$ cells

Dynabeads® Epithelial Enrich contains 4 × $10^8$ beads/mL in phosphate buffered saline (PBS), pH 7.4, with 0.1% bovine serum albumin (BSA) and 0.02% sodium azide as a preservative.
**Caution:** Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

## Product Description

Isolate or deplete human epithelial cells directly from whole blood, buffy coat, bone marrow, mononuclear cells (MNC) or tissue samples with Dynabeads® Epithelial Enrich. The enriched cells are viable and may be used in cellular applications or lysed for further molecular analysis. Circulating tumor epithelial cells are extremely rare and may be present at a frequency of only 1–10 per $10^6$ MNC and an enrichment step is usually essential to obtain a sufficient specificity and sensitivity for tumor cells.

Dynabeads® are mixed with the sample in a tube. The Dynabeads® will bind to the target cells during a short incubation, and then the bead-bound cells can subsequently be separated by a magnet.

**Depletion** – Discard the bead-bound cells and use the remaining, untouched cells for any application.

**Positive isolation** – Discard the supernatant and use the bead-bound epithelial cells for downstream applications.

## Downstream Applications

Epithelial cells can be efficiently depleted from a sample.

For rapid and consistent results in protein or gene expression analysis, lyse the epithelial cells while still attached to the beads and directly process for further molecular analysis e.g. mRNA isolation and RT-PCR.

For positive isolation for functional studies, or for flow cytometer analysis, the cells need to be released after isolation. For these applications, we recommend using Dynabeads® CELLection™ Epithelial Enrich (gives bead-free cells).

## Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Isolation Buffer: $Ca^{2+}$ and $Mg^{2+}$ free PBS pH 7.4 with 0.1% (w/v) BSA and 2 mM EDTA.
  **Note:** BSA can be replaced by human serum albumin (HSA) or fetal calf serum (FCS). EDTA can be replaced by sodium citrate.

## General Guidelines

- Visit www.lifetechnologies.com/samplepreparation for recommended sample preparation procedures.
- Use a mixer that provides tilting and rotation of the tubes to ensure that Dynabeads® do not settle in the tube.
- This product should not be used with the MPC™-1 magnet. (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Carefully follow the recommended pipetting volumes and incubation times.
- Keep all buffers cold.

## Protocol

### Wash Dynabeads®

See Table 1 and Table 2 for volume recommendations.

1. Resuspend the Dynabeads® in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of Dynabeads® to a tube.
3. Add the same volume of Isolation Buffer, or at least 1 mL, and resuspend.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed Dynabeads® in the same volume of Isolation Buffer as the initial volume transferred of Dynabeads® (step 2).

### Prepare Sample

- Cells can be directly isolated from any sample such as whole blood, bone marrow, MNC suspensions or tissue digests.
- Prepare MNC to 1 × $10^7$ cells/mL in Isolation Buffer at 2°C to 8°C.
- Use anti-coagulated whole blood (EDTA or ACD). Keep on ice.
- See "General Guidelines" for sample preparation procedures.

### Positively Isolate Tumor Cells from MNC

The protocol is based on 1 mL (1 × $10^7$) MNC as starting sample, but is scalable from 1 × $10^7$–5 × $10^8$ MNC. When working with lower volumes than 1 mL, use the same volumes as indicated for 1 mL. When working with larger volumes, scale up all volumes accordingly, as shown in Table 1.

1. Add 25 µL re-suspended and pre-washed Dynabeads® to 1 mL MNC.
2. Incubate for 30 min at 2°C to 8°C with gentle tilting and rotation.
3. Resuspend the bead-bound cells and place the tube in the magnet for 3 min at 2°C to 8°C. Invert the magnet with the tube gently after 1 minute, to collect beads left in the cap of the tube.
4. While the tube is still in the magnet, carefully pipette off and discard the supernatant.
5. Remove the tube from the magnet and add 1 mL cold Isolation Buffer.
6. Repeat steps 3–5 three times, i.e. a total of 4 washes.
7. Transfer the bead suspension to a new tube.

Keep on ice for immediate downstream applications

Table 1: Volumes for isolation/depletion of human epithelial cells from MNC. This protocol is scalable from 1 × $10^7$ to 5 × $10^8$ cells.

| Step | Step description | Volumes per 1 × $10^7$ MNC | Volumes per 1 × $10^8$ MNC |
|---|---|---|---|
| | Recommended tube size | 5 mL | 15 mL |
| | Recommended magnet | DynaMag™-5 | DynaMag™-15 |
| 1 | Volume MNC | 1 mL | 10 mL |
| 1* | Dynabeads® volume | 25 µL | 250 µL |
| 3–5 | Wash cells (Isolation Buffer) | 4 × ~1 mL | 4 × ~10 mL |

* If very high cell-depletion efficiency is required, increase the Dynabeads® volume up to double the recommended amount.

## Positively Isolate Tumor Cells from Whole blood and Buffy Coat

The protocol is based on 5 mL whole blood/buffy coat as starting sample, but is scalable from 5 mL– 40 mL. When working with lower volumes than 5 mL, use the same volumes as indicated for 5 mL. When working with larger volumes, scale up all volumes accordingly, as shown in Table 2.

1. Add 125 µL re-suspended and pre-washed Dynabeads® to 5 mL whole blood/buffy coat.
2. Incubate for 30 min at 2°C to 8°C with gentle tilting and rotation.
3. Place the tube in the magnet for 5 min at 2°C to 8°C. Invert the magnet with the tube gently after 3 min, in order to collect beads left in the cap of the tube.
4. While the tube is still in the magnet, carefully remove and discard the supernatant.
5. Add 1 mL cold Isolation Buffer while the tube is still on the magnet, in order to remove blood left in the tube and on the tube walls. Avoid disturbing the bead pellet.
6. Discard the supernatant. Remove the tube from the magnet and add 1 mL cold Isolation Buffer.
7. Resuspend the bead-bound cells carefully and transfer the suspension to a new tube.
8. Place the tube in the magnet for 5 min at 2°C to 8°C. Invert the magnet with the tube gently after 3 min, in order to collect beads left in the cap of the tube.
9. Repeat steps 6–8 three times, i.e. a total of 5 washes.
10. Transfer the bead suspension to a new tube.

Keep on ice for immediate downstream applications.

Table 2: Volumes for isolation/depletion of human epithelial cells from whole blood/buffy coat. This protocol is scalable from 5 mL to 40 mL .

| Step | Step description | Small scale (1X) | Large scale (8X) |
|---|---|---|---|
| | Recommended tube size | 5 mL | 50 mL |
| | Recommended magnet | DynaMag™-5 | DynaMag™-50 |
| 1 | Volume blood/buffy | 5 mL | 40 mL |
| 1* | Dynabeads® volume | 125 µL | 1 mL |
| 5 | Wash the tube (Isolation Buffer) | ~1 mL | ~8 mL |
| 3–5 | Wash cells (Isolation Buffer) | 4 × ~1 mL | 4 × ~10 mL |

* If very high cell-depletion efficiency is required, increase the Dynabeads® volume up to double the recommended amount.

## Description of Materials

Dynabeads® Epithelial Enrich are uniform, superparamagnetic, polystyrene beads (4.5 µm diameter) coated with a mouse IgG1 monoclonal antibody (clone Ber-EP4) specific for two (34 and 39 kDa) glycopolypeptide membrane antigens expressed on most normal and neoplastic human epithelial tissues. The reactivity of this antibody is identical to mAb clone HEA125.

## Related Products

| Product | Cat. no. |
|---|---|
| DynaMag™-5 | 12303D |
| DynaMag™-15 | 12301D |
| DynaMag™-50 | 12302D |
| HulaMixer® Sample Mixer | 15920D |
| Dynabeads® CELLection™ Epithelial Enrich | 15920D |

REF on labels is the symbol for catalog number.

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