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CELLection™ Epithelial Enrich

Catalog no. 16203 **Store at 2˚C to 8˚C**

Rev. Date: June 2012 (Rev. 003)

Kit Contents

Kit contents	Volume
CELLection™ Epithelial Enrich Dynabeads®	5 mL
Releasing Buffer Component 1 (DNase I)	2 vials
Releasing Buffer Component 2	1 mL

Kit capacity

Whole blood/buffy coat: 100 mL

Mononuclear cells (MNC): ~2 × 10^9

For product content details, see "Description of Materials" section.

Product Description

This product is intended for isolation of human epithelial cells directly from whole blood, bone marrow, MNC, or tissue digests. The enriched cells are bead-free, and may be cultured directly and used for immunocytochemical staining. For depletion of human epithelial cells or positive isolation for downstream molecular analysis, we recommend using Dynabeads® Epithelial Enrich.

CELLection™ Epithelial Enrich Dynabeads® are mixed with the cell sample in a tube. The Dynabeads® bind to the target cells during a short incubation, and the bead-bound cells are separated by a magnet. The beads are removed from the cells using the Releasing Buffer Component 1 (DNase I) (fig. 1).

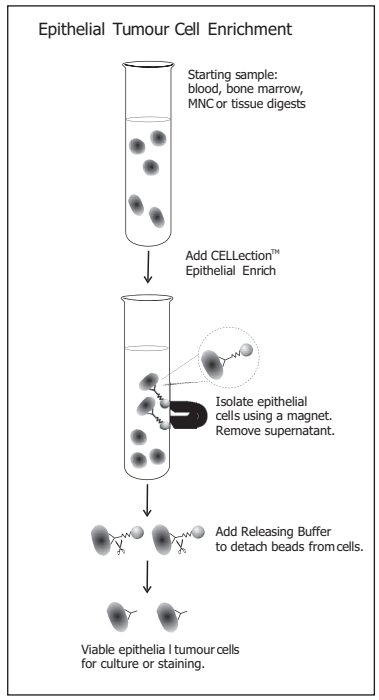

Figure 1: Overview of enrichment method

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Buffer 1: Ca^{2+} and Mg^{2+} free PBS supplemented with 0.1% BSA, pH 7.4.
- Buffer 2: Ca^{2+} and Mg^{2+} free PBS with 0.1% BSA and 0.6% Na-citrate or 2 mM EDTA.
  Note: BSA can be replaced by human serum albumin (HSA) or fetal calf serum (FCS).
- Buffer 3: RPMI 1640 with 1% fetal calf serum (FCS), 1 mM $CaCl_2$ and 5 mM $MgCl_2$, pH 7.0–7.4.
- *Optional:* DNase I 10.000–20.000 IU/mL.

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 at the bottom of 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.
- For optimal DNase I activity ensure that the pH in Buffer 3 is 7.0–7.4.

Protocol

Wash Dynabeads®

See Table 1 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 Buffer 1, 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 Buffer 1 as the initial volume of Dynabeads® (step 2).

Release Buffer Preparation

1. For each vial of freeze-dried DNase I, transfer 300 µL from the Releasing Buffer Component 2 to each vial of Releasing Buffer Component 1 (DNase I).
2. Dissolve the enzyme gently by tilting gently or pipetting up and down. Vigorous mixing will destroy the enzyme.
3. Aliquot the reconstituted Releasing Buffer into suitable portions. Store at –20°C. Thaw immediately before use and keep on ice once thawed. Thawed Releasing Buffer can be re-frozen once.

Prepare Sample

Wash whole blood and bone marrow

Important for removal of interfering factors.

1. Dilute the whole blood or bone marrow in Buffer 2 (1:1). Centrifuge at 600 × g for 10 min at room temperature.
2. Discard the plasma fraction/upper layer.
3. Resuspend to the original volume in Buffer 2 at 2°C to 8°C.

DNase treatment of bone marrow

Important for removal of interfering DNA.

1. Dilute washed bone marrow in Buffer 3 (1+4).
2. Add 120 IU DNase I/mL (not supplied).
3. Incubate for 30 min at room temperature with gentle tilting and rotation.
4. Centrifuge at 600 × g for 10 min at room temperature and discard the supernatant.
5. Resuspend cells in the same volume of Buffer 2.
6. Repeat step 4 once.
7. Resuspend at 2 × 10^7 cells/mL in Buffer 2 at 2°C to 8°C.

MNC preparation from whole blood, buffy coat or bone marrow

- Prepare MNC according to “General Guidelines”.
- Resuspend MNC at 2 × 10^7 cells/mL in Buffer 2 at 2°C to 8°C.

Enrich Tumor Cells from MNC or Whole Blood/Buffy Coat

The protocol is based on 1 mL (2×10^7) MNC or 5 mL whole blood or buffy coat as starting sample, but is scalable from 2×10^7–5×10^8 MNC or 5–50 mL whole blood/buffy coat. When working with lower volumes than shown for small scale (1X), use the same volumes as indicated for small scale (1X). When working with larger volumes, scale up all reagent and volumes accordingly, as shown in Table 1.

1. Add 50 µL Dynabeads® to 1 mL MNC, or 250 µL 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 a magnet for 2 min.
4. While the tube is still in the magnet, carefully remove and discard the supernatant
5. Remove the tube from the magnet and add 1 mL Buffer 1 (for MNC) or 5 mL Buffer 1 (for blood/buffy), pipet 2–3 times (or vortex 2–3 sec) and place the tube in a magnet for 2 min.
6. Repeat steps 4–5 at least twice to wash the bead-bound cells. This step is critical to obtain a high purity of isolated cells.
7. Resuspend the bead-bound cells in 200 µL Buffer 3 preheated to 37°C. Proceed to "Release of Tumor Cells".

Release Tumor Cells

Before transferring the released cells to a new tube, pre-coat the tubes for 5 min using Buffer 3.

8. Add 4 µL reconstituted Release Buffer (DNase I).
9. Incubate for 15 min at room temperature with gentle tilting and rotation.
10. Pipet thoroughly with a 100–200 µL pipette at least 5–10 times to maximize cell release (avoid foaming).
11. Place in a magnet for 2 min and transfer the supernatant with released cells into a pre-coated tube.
12. Resuspend the bead fraction in 200 µL Buffer 3 and repeat steps 10–11 once.

The cells are now bead-free and ready for use in any downstream applications.

Controls

Samples from healthy donors spiked with epithelial cells (e.g. SW480, ATCC no. CCL-228) can be used as positive control for the enrichment protocol.

Table 1: Volumes for isolation of human epithelial cells from MNC or whole blood/buffy coat.

Step	Step description	Small scale (1X)	Large scale (10X)
	Recommended tube size	5 mL	15 mL
	Recommended magnet	DynaMag™-5	DynaMag™-15
1*	Volume MNC /volume blood of buffy	1 mL 5 mL	10 mL 50 mL
1	Dynabeads®	50 µL (MNC) 250 µL (blood/buffy	500 µL (MNC) 2.5 mL (blood/buffy)
4	Wash beads (Buffer 1)	1 mL × 3 (MNC) 5 mL × 3 (blood/buffy)	10 mL × 3 (MNC) 30 mL × 3 (blood/buffy)
5*	Resuspend beads (Buffer 2)	200 µL	2 mL
6	Release cells (Release Buffer)	4 µL	40 µL
10	Collect residual cells (Buffer 3)	2 × 200 µL	2 × 2 mL

* 2×10^7 cells/mL.

Description of Materials

CELLection™ Epithelial Enrich Dynabeads® are uniform, superparamagnetic polymer beads (4.5 µm diameter) coated with a monoclonal mouse IgG1 antibody (Ber-EP4) via a DNAlinker to provide a cleavage site for cell release. Ber-EP4 is an anti-EpCAM (epithelial cell adhesion molecule) antibody with the same reactivity as HEA125, and is specific for two lycopolypeptide membrane antigens expressed on most normal and neoplastic human epithelial cells. The Releasing Buffer Component I contains freeze-dried DNase 1 and needs to be reconstituted in Releasing Buffer Component II prior to use.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
Dynabeads® Epithelial Enrich	16102
HulaMixer® Sample Mixer	15920D

REF on labels is the symbol for catalog number.

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