invitrogen
by life technologies

Dynabeads® CD45

Catalog no. 11153D

Store at 2˚C to 8˚C

Rev. Date: March 2012 (Rev. 003)

Product Content

Product contents	Volume
Dynabeads® CD45	5 mL

Product capacity
Whole blood: ~50 mL
Positive isolation: ~5 x 10^8 MNC
Tumor cell enrichment: ~2 × 10^8 MNC

Dynabeads® CD45 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 $CD45^+$ cells directly from whole blood, buffy coat, or mononuclear cells (MNC) with Dynabeads® CD45. For rapid and consistent results in protein or gene expression analysis, lyse the leucocytes while they are still attached to the beads and directly process for further molecular analysis. The beads are mixed with the cell sample in a tube. The beads bind to the target cells during a short incubation, and then the bead-bound cells are separated by a magnet (fig. 1).

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 cells for downstream applications.

Downstream Applications

$CD45^+$ cells can be efficiently depleted from a sample to, for example, enrich for circulating tumor cells (non-hematopoetic) or positively isolated for molecular downstream applications. For positive isolation for functional studies, or for flow cytometer analysis, the cells need to be released after isolation. For this, we recommend using Dynabeads® FlowComp™ Flexi with your own CD45 antibody (bead-free cells).

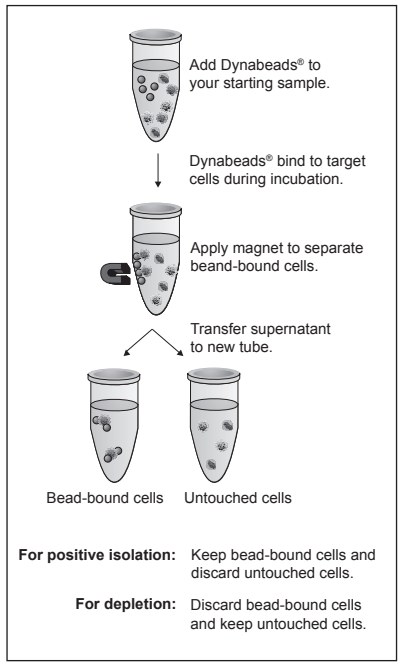

Figure 1: Overview of method

Required Materials

- Magnet (DynaMag™ portfolio). See www.lifetechnologies.com/magnets for recommendations.
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Isolation Buffer 1: Ca^{2+} and Mg^{2+} free PBS supplemented with 0.1% BSA and 2 mM EDTA, pH 7.4.
  Note: BSA can be replaced by human serum albumin (HSA) or fetal calf serum (FCS). EDTA can be replaced by sodium citrate.
- Isolation Buffer 2: RPMI 1640 supplemented with 1% fetal calf serum (FCS), 1 mM $CaCl_2$ and 4 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 beads 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 the Beads

See Table 1 and Table 2 for volume recommendations.

1. Resuspend the beads in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of beads to a tube.
3. Add the same volume of Isolation 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 beads in the same volume of Isolation Buffer 1 as the initial volume transferred of beads (step 2).

Prepare Cells

- Cells can be directly isolated from any sample such as whole blood, bone marrow, MNC suspensions or tissue digests.
- See “General Guidelines” for sample preparation procedures.
- Prepare MNC from whole blood/buffy coat to 2×10^7 cells/mL in Isolation Buffer 1 at 2°C to 8°C.
- Prepare MNC from bone marrow (BM-MNC) to 2×10^7 cells/mL in Isolation Buffer 2 at 18°C to 25°C. BM-MNC should be DNAse treated to remove interfering DNA prior to isolation.

DNase Treatment of BM-MNC

BM-MNC should be frozen in the presence of 120 IU DNase solution/mL cell suspension.

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

Enrich Tumor Cells from MNC

This protocol is specific for enrichment of tumor cells from MNC by CD45 depletion. Before cell isolation or transferring the enriched cells to a new tube, pre-coat the tubes for 5 min using buffer with FCS (e.g. Isolation Buffer 2). This protocol is based on 1 mL (2×10^7) MNC, but is scalable from 2×10^7–5×10^8. 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 250 µL pre-washed and re-suspended beads to the pre-coated tube.
2. Add 1 mL prepared MNC to the beads and incubate for 30 min at 2°C to 8°C with gentle tilting and rotation.
3. Remove the tube from the mixer, transfer to a larger tube and dilute the sample 40 times with Isolation Buffer 1. Mix gently.
4. Place the tube in a magnet for 10 min.
5. Transfer the supernatant to a new pre-coated tube. Fill the tube to 50 mL with Isolation Buffer 1.
6. Centrifuge at 600 × g for 15 min at 2°C to 8°C and resuspend the cells in an appropriate volume of Isolation Buffer 2 depending on the downstream assay.

Enriched tumour cells can be detected and analysed by immunocytochemistry (ICC).

Table 1: Volumes for tumor cell enrichment from MNC. This protocol is scalable from 2×10^7 to 5×10^8 cells.

Step	Step description	Volumes per 2×10^7 MNC	Volumes per 2×10^8 MNC
	Recommended tube size	5 mL/50 mL	15 mL
	Recommended magnet	DynaMag™-50	DynaMag™-50
1	Bead volume	250 µL	2.5 mL
1*	Cell volume	1 mL	10 mL
3**	Dilute cells (Isolation Buffer 1)	~40 mL	~35 mL
5**	Increase volume	up to 50 mL	up to 50 mL

* 2×10^7 cells/mL. Use a small tube for the bead-cell mixing step.
** Adjust the Isolation Buffer volumes to fit to the tube you are using.

Deplete or Positively Isolate CD45⁺ Cells

This general protocol is based on 1 mL (1×10^7) MNC, 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 2.

1. Transfer 1 mL cells (1×10^7) to a tube and add 100 µL pre-washed and re-suspended beads.
2. Incubate for 20 min (positive isolation) or 30 min (depletion) at 2°C to 8°C with gentle tilting and rotation.
3. *Optional:* increase the volume with 1 mL Isolation Buffer 1 to limit trapping of unbound cells.
4. Place the tube in a magnet for 2 min.
5. For *depletion;* transfer supernatant to a new tube for further use and discard the beads.
   or
   For *positive isolation;* while the tube is still in the magnet, carefully remove and discard the supernatant.
6. Remove the tube from the magnet and add 1 mL Isolation Buffer, pipet 2–3 times (or vortex 2–3 sec) and place the tube in a magnet for 2 min. While the tube is still in the magnet, carefully remove and discard the supernatant.
7. Repeat step 6 at least once to wash the bead-bound CD45⁺ cells. This step is critical to obtain a high purity of isolated cells.
8. Resuspend the cell pellet in preferred cell medium.

Keep the cells on 2°C to 8°C until further use in downstream applications.

Table 2: Volumes for positive isolation/depletion of CD45⁺ cells from MNC. This protocol is scalable from 1×10^7 to 4×10^8 cells.

Step	Step description	Volumes per 1×10^7 MNC	Volumes per 1×10^8 MNC
	Recommended tube size	5 mL	50 mL
	Recommended magnet	DynaMag™-5	DynaMag™-50
1*	Cell volume	1 mL	10 mL
1	Bead volume	100 µL	1 mL
3	Dilute cells (Isolation Buffer 1) - optional:	~1 mL	~10 mL
6–7**	**For positive isolation only:** Wash cells (Isolation Buffer)	4 × ~1 mL	4 × ~10 mL

* 1×10^7 cells/mL.
** Adjust the Isolation Buffer 1 volume to fit to the tube you are using.

Description of Materials

Dynabeads® CD45 are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with a primary monoclonal mouse IgG2a antibody specific for a CD45 membrane antigen common to all known isoforms of CD45. CD45 is expressed on all human leucocytes.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D
Dynabeads® FlowComp™ Human Flexi Kit	11061D

REF on labels is the symbol for catalog number.

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