invitrogen
by life technologies

Dynabeads® CD15

Catalog no. 11137D **Store at 2˚C to 8˚C**

Rev. Date: May 2012 (Rev. 006)

Product Content

Product contents	Volume
Dynabeads® CD15	5 mL

Product capacity
Whole blood: 100 mL
MNC: ~2 × 10^9 cells

Dynabeads® CD15 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 $CD15^+$ myeloid cells (predominantly neutrophil and eosinophil granulocytes) directly from whole blood, buffy coat, or MNC with Dynabeads® CD15. For rapid and consistent results in protein or gene expression analysis, lyse the granulocytes 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 molecular applications.

Downstream Applications

$CD15^+$ cells can be efficiently depleted from a sample. For rapid and consistent results in protein or gene expression analysis, lyse the $CD15^+$ T cells while still attached to the beads and directly process for further molecular analysis. For positive isolation for functional studies, cell activation/expansion, or for flow cytometer analysis, the cells need to be released after isolation. For this, we recommend using Dynabeads® FlowComp™ Flexi with your own CD15 antibody (bead-free cells).

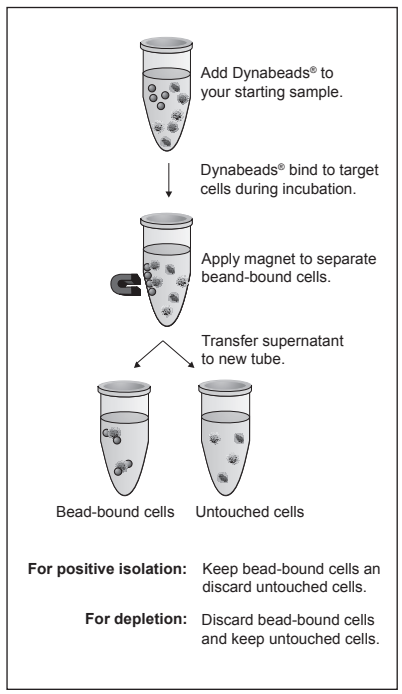

Figure 1: Overview of method

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: 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.

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 MPC™-1 (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 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, 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 as the initial volume 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. Whole blood and buffy coat need to be washed prior to isolation.
- Prepare MNC to 1 × 10^7 cells/mL in Isolation Buffer.
- See "General Guidelines" for sample preparation procedures.

Dilute Whole Blood and Buffy Coat

Blood and buffy coat can be diluted before use to decrease the granulocyte concentration. This is to ensure sufficient number of beads per target in granulocyte-rich samples. Beads can be added directly to undiluted blood or buffy coat if reduced cell isolation efficiency is tolerated (e.g. rapid isolation for molecular studies).

1. Dilute whole blood (1:2) in Isolation Buffer.
2. Dilute buffy coat (1:4) in Isolation Buffer.

Deplete or Positively Isolate CD15⁺ Cells

The protocol is based on 1 mL (1×10^7) MNC or 1 mL diluted whole blood/buffy coat as starting sample, but is scalable from 1×10^7–5×10^8 (1–50 mL).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 reagent and volumes accordingly, as shown in Table 1.

1. Transfer 1 mL cells (1×10^7) to a tube and add 25 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. Place the tube in a magnet for 2 min.
4. For *depletion*; transfer supernatant to a new tube for further use and discard the beads.
5. For *positive isolation*; while the tube is still in the magnet, carefully pipet off 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 seconds) and place the tube in a magnet for 2 min.
7. Repeat steps 5–6 at least once to wash the bead-bound CD15⁺ cells. These steps are 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 1: Volumes for isolation/depletion of human CD15⁺ cells. This protocol is scalable from 1×10^7 to 5×10^8 cells.

Step	Step description	Small scale (1X)	Large scale (10X)
	Recommended tube size	5 mL	15 mL
	Recommended magnet	DynaMag™-5	DynaMag™-15
1	Sample volume (MNC*/blood/buffy)	1 mL	10 mL
1**	Bead volume	25 µL	250 µL
5–7	Wash cells (Isolation Buffer)	3 × ~1 mL	3 × ~10 mL

* 1×10^7 cells/mL.

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

Description of Materials

Dynabeads® CD15 are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with a primary monoclonal mouse IgM antibody specific for the CD15 membrane antigen, which is predominantly expressed on human neutrophil and eosinophil granulocytes, and to a varying degree on monocytes. The CD15 antigen shows heterogeneous expression on normal myeloid precursor cells, myeloid leukemias, myeloid cell lines and Sternberg-Reed cells. The CD15 antigen is widely distributed outside the hematopoietic system.

Related Products

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

REF on labels is the symbol for catalog number.

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