invitrogen
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Dynabeads® CD14

Catalog no. 11149D **Store at 2˚C to 8˚C**

Rev. Date: March 2012 (Rev. 003)

Product Contents

Product contents	Volume
Dynabeads® CD14	5 mL

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

Dynabeads® CD14 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 $CD14^+$ monocytes directly from whole blood, buffy coat or MNC with Dynabeads® CD14. 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

$CD14^+$ cells can be efficiently depleted from a sample. For rapid and consistent results in protein or gene expression analysis, lyse the $CD14^+$ 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™ Human CD14 (bead-free cells).

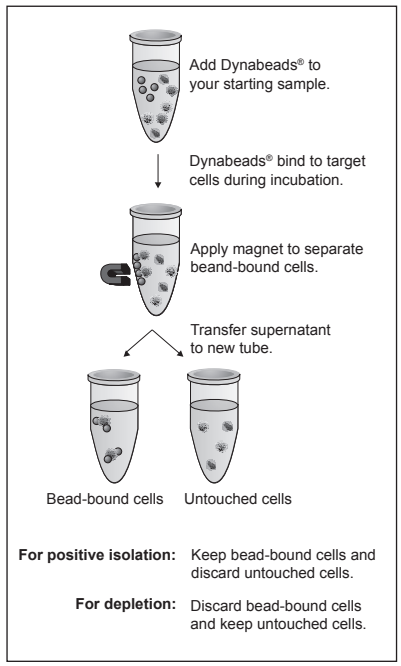

Figure 1: Overview of method

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.
- It is very important to wash whole blood and buffy coat prior to isolation, to ensure high recovery/depletion efficiency.
- 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.

Protocols

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.

Wash Whole Blood and Buffy Coat

Wash the blood/buffy coat to remove soluble CD14.

1. Dilute the whole blood or buffy coat in Isolation Buffer 1 (1:2).
2. Centrifuge at 600 × g for 10 min at room temperature.
3. Discard the plasma fraction/upper layer.
4. Resuspend blood/buffy coat to the original volume in Isolation Buffer before adding the beads.

Deplete or Positively Isolate CD14⁺ Cells

The protocol is based on 1 mL (1×10^7) MNC or 1 mL whole blood/buffy coat as starting sample, but is scalable from $1 \times 10^7 – 5 \times 10^8$ (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 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.
   or
   For *positive isolation*; 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 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.
6. Repeat step 5 at least once to wash the bead-bound CD14⁺ cells. This step is critical to obtain a high purity of isolated cells.
7. 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 CD14⁺ 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
5–6	**For positive isolation only:** 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® CD14 are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with a primary monoclonal antibody specific for the CD14 membrane antigen expressed on most human monocytes.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
HulaMixer® Sample Mixer	15920D
Dynabeads® FlowComp™ Human CD14	11367D

REF on labels is the symbol for catalog number.

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