invitrogen
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Dynabeads® CD31 Endothelial Cell

Catalog no. 11155D **Store at 2˚C to 8˚C**

Rev. Date: March 2012 (Rev. 004)

Product Contents

Product Capacity	Volume
Dynabeads® CD31 Endothelial Cell	5 mL

Product capacity
~2 × 10^{10} cells

Dynabeads® CD31 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 CD31⁺ human microvessel endothelial cells directly from prepared tissue digests with Dynabeads® CD31. This product can also be used for re-selection of endothelial cells grown in culture. Dynabeads® CD31 bind human umbilical vein endothelial cells (HUVEC). Microvessel endothelial cells have been isolated from many human tissues including adipose (1–4), brain (12–15), dermal (16–19), HUVEC (16, 26, 27), endometrial (20), gastric and intestinal (21, 22), heart (23–25), liver (28), lung (29–31), placenta (32, 33), renal (34), synovial (16, 35) and tonsil (36) tissue. CD31 is also expressed on platelets and white blood cells such as monocytes, NK cells, granulocytes, B cells and T cell subsets, thus Dynabeads® CD31 cannot be used directly on whole blood or bone marrow samples. 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.

Positive isolation - Discard the supernatant and use the bead-bound endothelial cells for downstream applications. Positively isolated endothelial cells are pure, viable, and un-stimulated and are ideal for culture with the beads still attached. Fibroblast, pericyte or smooth muscle cell contamination is avoided. The cells grow well and adhere normally and after approximately three passages, all the beads are diluted out. For rapid and consistent results in protein or gene expression analysis, lyse the CD31⁺ cells while they are still attached to the beads and directly process for further molecular analysis

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

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: PBS w/0.1% BSA, pH 7.4.
- Buffer 2: PBS w/0.25% trypsin and 1 mM EDTA.
- Buffer 3: PBS w/5 % fetal calf serum (FCS).

General Guidelines

- 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 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 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 Buffer 1 as the initial volume transferred of beads (step 2).

Prepare Cells

Prepare a single cell suspension and resuspend to 1 × 10^8 cells/mL in Isolation Buffer. See "References" section for references for different tissue preparation procedures.

Positively Isolate or Deplete CD31⁺ Endothelial Cells

The protocol is based on 1 × 10^8 cells (1 mL) (single cell suspensions prepared from tissue) as starting sample, but is scalable from 1 × 10^8 – 5 × 10^9. 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 prepared cells (1 × 10^8) to a tube and add 25 uL pre-washed and re-suspended Dynabeads.
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 Buffer 1, 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 CD31⁺ endothelial cells. This step is critical to obtain a high purity of isolated cells.
7. Resuspend the cell pellet in preferred cell medium.

Table 1: Volumes for isolation/depletion of human CD31⁺ endothelial cells. This protocol is scalable from 1 × 10^8 to 5 × 10^9 cells.

Step	Step description	Small scale (1X)	Large scale (10X)
	Recommended tube size	5 mL	15 mL
	Recommended magnet	DynaMag™-5	DynaMag™-15
1	Cell volume	1 mL	10 mL
1*	Bead volume	25 µL	250 µL
5–6	Wash cells (Isolation Buffer)	3 × ~1 mL	3 × ~10 mL

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

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Re-select Endothelial Cells from Cultures

1. Remove medium from the culture dish with the grown endothelial cells.
2. Add 0.5 mL Buffer 2 (35 mm petri dish), incubate for 5 min at 37°C, knock the dish to dislodge the cells (check by microscope).
3. Add 2 mL Buffer 3.
4. Wash once by centrifugation and resuspend endothelial cells to < 2 × 10^6/mL in Buffer 1.
5. Add 25 µL washed beads per mL cell suspension.
6. Incubate for 20 min at 2°C to 8°C with gentle tilting and rotation.
7. Increase the volume two fold with Buffer 1 and place the tube in a magnet for 2 min.
8. Discard the supernatant and wash the bead-bound cells 3 times by re-suspending in Buffer 1 to the same volume as discarded, and separate using a magnet.
9. The bead-bound endothelial cells are now ready for plating or further analysis.

Description of Materials

Dynabeads® CD31 are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with a mouse IgG1 monoclonal antibody specific for the CD31 cell surface antigen PECAM-1 (platelet endothelial cell adhesion molecule-1). The primary CD31 antibody is attached to the beads via a secondary human anti-mouse IgG antibody to ensure optimal orientation of the primary antibody.

Related Products

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

REF on labels is the symbol for catalog number.

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