invitrogen
by life technologies

Dynabeads® Sheep anti-Rat IgG

Catalog no. 11035 **Store at 2˚C to 8˚C**

Rev. Date: May 2012 (Rev. 004)

Product Contents

Product contents	Volume
Dynabeads® Sheep anti-Rat IgG	5 mL

Maximum product capacity

MNC*: ~2 × 10^9 cells

Whole blood/buffy coat: ~200 mL

*Note: If using the product for negative isolation of multiple cell types simultaneously, the bead volume used is higher, thus giving a lower product capacity (see Table 1 and 2).

Dynabeads® Sheep anti-Rat IgG contains 4 × 10^8 Dynabeads®/mL in phosphate buffered saline (PBS), pH 7.4, containing 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

Dynabeads® Sheep anti-Rat IgG in combination with primary rat IgG antibodies are ideal for depletion or positive isolation of cells from different species (e.g. mouse, human), depending on the specificity of the primary antibody. Cells can be directly isolated from any sample such as whole blood, bone marrow, MNC suspensions or tissue digests.

The primary rat IgG antibodies are either added to the cell sample (indirect technique) or pre-coated onto the beads (direct technique) prior to cell isolation Dynabeads® are then mixed with the cell sample in a tube. The Dynabeads® 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 cells for downstream applications (e.g. isolation of proteins, nucleic acids (NA) or cell culture).

Note: For positive isolation of cells for downstream cellular applications or for use in flow cytometry, bead-free cells are required. For these applications, use Dynabeads® FlowComp Flexi (to obtain bead-free cells). The kit contains biotinylation and release reagents. Standard biotinylated antibodies will not give release with that kit.

Depletion/negative isolation – Discard the bead-bound cells and use the remaining bead-free and untouched cells for any application. Different rat IgG antibodies can be used to deplete several cell types simultaneously (negative isolation) to obtain untouched cells.

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 phosphate buffered saline (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.
- Rat IgG antibodies.

General Guidelines

- Visit www.lifetechnologies.com/samplepreparation for recommended sample preparation procedures.
- The choice of primary antibody is the most important factor for successful cell isolation. Note that some antibodies may show reduced antigen-binding efficiency when coated onto beads, even though the antibody shows good results in other immunological assays.
- To avoid non-specific binding of cells (e.g. monocytes, B cells), add aggregated IgG to block Fc-receptors prior to adding the primary antibodies.
- Wash cells prior to adding rat IgG antibodies or Dynabeads® to remove density gradient media (e.g. Ficoll) or soluble factors in serum (e.g. antibodies or cell surface antigens), which can interfere with the cell isolation protocol.
- Use a mixer that provides tilting and rotation of the tubes to ensure that Dynabeads® 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.
- Never use less than recommended volume of Dynabeads®.
- Carefully follow the recommended pipetting volumes and incubation times.
- Keep all buffers cold.

Indirect versus Direct Technique

Use the indirect technique when: A cocktail of rat monoclonal antibodies is used to deplete several cell types simultaneously (use MNC as a starting sample to remove erythrocytes, platelets and granulocytes), very high depletion efficiency is required, the affinities of rat antibodies are low, the cells express low number of target antigens or the direct technique gives unsatisfactory purity.

Use the direct technique when: The affinity of the primary antibody is high, the cells express a high number of target antigens or to make a larger stock preparation of primary coated Dynabeads® (will generally have the same shelf life as stated on the Dynabeads® vial).

Protocol

Wash Dynabeads®

See Table 1 and 2 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 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 Dynabeads® in the same volume of Isolation Buffer as the initial volume of Dynabeads® (step 2).

Prepare Cells

Cells can be directly isolated from any samples such as whole blood, bone marrow, MNC suspensions, or tissue digests. See "General Guidelines" for sample preparation.

- Prepare a MNC suspension according to "General Guidelines". Resuspend the cells at 1 × 10^7 cells/mL in Isolation Buffer.
- See “General Guidelines” for recommendation of when to use the direct vs. indirect cell isolation technique.
- This protocol is based on 1 × 10^7 MNC or 1 mL whole blood, but is directly scalable from 1 × 10^7 to 4 × 10^8 cells or 1–40 mL whole blood. When working with lower volumes than 1 × 10^7 cells or 1 mL blood, use the same volumes as for 1 × 10^7 cells or 1 mL blood. When working with larger volumes, scale up all volumes accordingly, as shown in Table 1 and 2.
  Note: When doing negative isolation it is recommended to isolate the cells from a prepared MNC sample rather than from whole blood to remove erythrocytes, platelets and granulocytes.

Isolate Cells – Indirect Technique (labeling cells with rat IgG antibodies)

Use approximately 10 µg of primary antibody (rat IgG) per 10^7 target cells. Titrate the primary antibody to optimize the amount used.

1. Add ~10 µg primary antibody to 1 mL cell suspension and mix (titrate the antibody amount for your use).
2. Incubate for 10 min at 2°C to 8°C.
3. Wash the cells by adding 2 mL Isolation Buffer and centrifuge at 350 × g for 8 min. Discard the supernatant.
4. Resuspend the cells in Isolation Buffer back to 1 × 10^7 MNC/mL (or 1 mL for blood).
5. For positive isolation or depletion of one cell type – add 25 µL pre-washed and resuspended Dynabeads®. For negative isolation (removal of multiple cell types simultaneously) add 100 µL Dynabeads®.

6. Incubate for 20 min (positive isolation) or 30 min (depletion/negative isolation) at 2°C to 8°C with gentle tilting and rotation.
7. *Optional:* Add 1 mL Isolation Buffer to limit trapping of unbound cells.
8. Place the tube in a magnet for 2 min.
9. *Depletion/negative isolation:* Transfer the supernatant containing the unbound cells to a fresh tube for further experiments.
   or
   Positive isolation: While the tube is still in the magnet, carefully remove and discard the supernatant.
10. 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.
11. Repeat steps 10–11 at least twice to wash the cells. These steps are critical to obtain a high purity of isolated cells.
12. Resuspend the cell pellet in preferred cell medium.

Table 1: Volumes for indirect cell isolation.

Step	Step description	Volumes per 1×10^7 MNC	Volumes per 2×10^8 MNC
	Recommended tube	5–7 mL tubes	50 mL tubes
	Recommended magnet	DynaMag™-5	DynaMag™-50
1	Primary rat IgG antibody	~10 µg	~200 µg
1	Cell volume (MNC/blood)	1 mL	20 mL
3*	Wash cells (Isolation Buffer)	~2 mL	~40 mL
4	Resuspend cells	1 mL	20 mL
5**(*)	Add Dynabeads® (positive isolation/depletion) Add Dynabeads® (negative isolation)	25 µL 100 µL	500 µL 2 mL
7*	Increase volume (Isolation Buffer)	~1 mL	~ 20 mL
10-12*	**For positive isolation only:** Wash the cells (Isolation Buffer)	3 × ~1 mL	3 × 20 mL

* Adjust the Isolation Buffer volumes to fit to the tube you are using.

** If very high depletion-efficiency is required or you are depleting many cells simultaneously, increase/optimize the amount of Dynabeads®.

*** When incubating, tilt and rotate the vial so the cells and beads are kept in the bottom of the tube. Do not perform end-over-end mixing if the volume is small relative to the tube size.

Isolate Cells – Direct Technique (antibody-coating of Dynabeads®)

Use 0.5–1.5 µg of primary rat IgG antibody per 25 µL (1×10^7) Dynabeads®. Titrate the primary antibody to optimize the amount used.

1. Transfer 25 µL pre-washed and resuspended Dynabeads® to a tube.
2. Add ~1 µg antibodies (titrate the antibody amount for your use).
3. Incubate for ≥30 minutes at 2°C to 8°C with gentle tilting and rotation.
4. Place the tube in a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and add 2 mL Isolation Buffer.
6. Repeat step 4–5 once to remove excess of antibodies.
7. Place the tube in the magnet for 1 min, discard the supernatant, remove the tube from the magnet and resuspend the Dynabeads® in 1 mL Isolation Buffer.
8. Add the beads to 1 mL cell sample (1×10^7 cells) and resuspend.
9. Incubate for 20 min (positive isolation) or 30 min (depletion) at 2°C to 8°C with gentle tilting and rotation.
10. *Optional:* Add 1 mL Isolation Buffer to limit trapping of unbound cells.
11. Place the tube in a magnet for 2 min.
12. *Depletion:* Transfer the supernatant containing the unbound cells to a fresh tube for further experiments.
    or
    Positive isolation: While the tube is still in the magnet, carefully remove and discard the supernatant.
13. 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.
14. Repeat steps 13–14 at least twice to wash the cells. These steps are critical to obtain a high purity of isolated cells.
15. Resuspend the cell pellet in preferred cell medium.

Table 2: Volumes for direct cell isolation.

Step	Step description	Volumes per 1×10^7 MNC	Volumes per 2×10^8 MNC
	Recommended tube	5–7 mL tubes	50 mL tubes
	Recommended magnet	DynaMag™-5	DynaMag™-50
1	Dynabeads®	~25 µL	~500 µL
2	Primary rat IgG antibody	~1 µg	~20 µg
5–6	Wash Dynabeads® (Isolation Buffer)	2 × ~2 mL	2 × ~40 mL
7	Resuspend Dynabeads® (Isolation Buffer)	1 mL	20 mL
8	Cell volume	1 mL	20 mL
10*	**Optional:** Increase volume (Isolation Buffer)	~1 mL	~8 mL
13–14*	**For positive isolation only:** Wash the cells (Isolation Buffer)	3 × ~1 mL	3 × 20 mL

* Adjust the Isolation Buffer volumes to fit to the tube you are using.

** If very high depletion-efficiency is required or you are depleting many cells simultaneously, increase/optimize the amount of Dynabeads®.

*** When incubating, tilt and rotate the vial so the cells and beads are kept in the bottom of the tube. Do not perform end-over-end mixing if the volume is small relative to the tube size.

Description of Materials

Dynabeads® Sheep anti-Rat IgG are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with polyclonal sheep anti-rat IgG antibodies. Cross-reactivity to mouse antibodies is high and cross-reactivity to human antibodies is minimal.

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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