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CELLection™ Pan Mouse IgG Kit

Catalog no. 11531D **Store at 2˚C to 8˚C**

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Kit Contents

Kit contents	Volume
CELLection™ Pan Mouse IgG Dynabeads®	5 mL
Releasing Buffer Component 1	3 vials
Releasing Buffer Component 2	2 mL

Kit capacity

Whole blood/buffy coat: ~200 mL

Mononuclear cells (MNC): ~2 × 10^9

CELLection™ Pan Mouse IgG Dynabeads® contains 4 × 10^8 beads/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

CELLection™ Pan Mouse IgG in combination with primary mouse IgG antibodies are ideal for positive isolation of cells from different species (e.g. human, rat) 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 enriched cells, which are bead-free and viable, may be used in any downstream application.

The mouse IgG primary antibody is either added to the cell sample (indirect technique) or pre-coated onto the beads (direct technique) prior to cell isolation. Dynabeads® magnetic beads are then mixed with the cell sample. The Dynabeads® magnetic beads bind to the target cells during a short incubation, and then the bead-bound cells are separated by a magnet. The beads are removed from the cells using the Releasing Buffer Component 1 (DNase I).

Note: For efficient depletion of cells, or positive isolation for downstream molecular analysis, please use the equivalent Dynabeads® Pan Mouse IgG product (no release of cells from the beads).

Note: It is not recommended to isolate phagocytic cells with this product. The cells will engulf the beads and there will be no detachment. For this purpose, use Dynabeads® FlowComp Flexi (can be used with lower temperature that is necessary to reduce phagocytic activity).

Required Materials

- DynaMag™ Magnet (See www.lifetechnologies.com/magnets for recommendations).
- Mixer allowing tilting and rotation of tubes (e.g. HulaMixer® Sample Mixer).
- Mouse IgG primary antibodies.
- Buffer 1: Ca^{2+} and Mg^{2+} free PBS supplemented with 0.1% bovine serum albumin (BSA), pH 7.4.
- Buffer 2: Ca^{2+} and Mg^{2+} free PBS with 0.1% BSA and 0.6% sodium citrate or 2 mM EDTA.
  Note: BSA can be replaced by human serum albumin (HSA) or fetal calf serum (FCS).
- Buffer 3: RPMI 1640 with 1% fetal calf serum (FCS), 1 mM $CaCl_2$ and 5 mM $MgCl_2$, pH 7.0–7.4.

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 Dynabeads® magnetic beads do not settle at the bottom of the tube.
- 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.
- Titrate the primary antibody to optimize the antibody amount used.
- Cell staining prior to flow cytometry: In the direct technique the cells can be stained with the same antibody as used for isolation, but this must be done before adding the Release Buffer (add fluorochrome conjugated antibody for 10 min prior to adding the Release Buffer).
- Blood and serum may contain soluble factors (e.g. antibodies or cell surface antigens), which can interfere with the cell isolation protocol. Washing the cells once may reduce this interference.
- This product should not be used with the MPC™-1 magnet (Cat. no. 12001D).
- Avoid air bubbles (foaming) during pipetting.
- Carefully follow the recommended volumes and incubation times.
- For optimal DNase I activity ensure that the pH in Buffer 3 is 7.0–7.4.

Indirect versus Direct Technique

- Use the *indirect technique* when very high depletion efficiency is required, the affinities of the primary 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® magnetic beads (will generally have the same shelf life as that stated on the Dynabeads® vial).

Protocols

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® magnetic 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 magnetic beads in the same volume of Buffer 1 as the initial volume of Dynabeads® magnetic beads (step 2).

Prepare Release Buffer

1. For each vial of freeze-dried DNase I, transfer 300 µL from the Releasing Buffer Component 2 to each tube of Releasing Buffer Component 1 (DNase I).
2. Dissolve the enzyme gently. Vigorous mixing will destroy the enzyme.
3. Aliquot the reconstituted Release Buffer into suitable portions. Store at –20°C. Thaw immediately before use and keep on ice once thawed. Thawed Release Buffer can be re-frozen once.

Prepare Sample

Wash whole blood and bone marrow

Important for removal of interfering factors.

1. Dilute the whole blood or bone marrow in Buffer 2 (1:2). Centrifuge at 600 × *g* for 10 min at room temperature.
2. Discard the plasma fraction/upper layer.
3. Resuspend to the original volume in Buffer 2 at 2°C to 8°C.

DNase treatment of bone marrow

Important for removal of interfering DNA. Visit www.lifetechnologies.com/samplepreparation for recommended DNA treatment procedures.

MNC preparation from whole blood, buffy coat, or bone marrow.

- Prepare MNC according to "General Guidelines".
- Resuspend MNC at 1 × 10^7 cells/mL in Buffer 2 at 2°C to 8°C.

Isolate Cells - Indirect Technique (Labeling Cells with Antibodies)

- See "General Guidelines" for recommendation of when to use the direct vs. indirect cell isolation technique.
- Use approximately 10 µg of primary mouse IgG antibody per 10^7 target cells.

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 reagents and volumes accordingly, as shown in Table 1.

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 Buffer 2 and centrifuge at 350 × g for 8 min. Discard the supernatant.
4. Resuspend the cells in Buffer 2 to 1 × 10^7 MNC per mL (or 1 mL for blood).
5. Add 25 µL pre-washed and resuspended Dynabeads® magnetic beads.
6. Incubate for 20 min at 2°C to 8°C with gentle tilting and rotation.
7. *Optional:* Add 1 mL Buffer 2 to limit trapping of unbound cells.
8. Place the tube in a magnet for 2 min.
9. 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 Buffer 1, pipet 2–3 times (or vortex 2–3 seconds) and place the tube in a magnet for 2 min.
11. Repeat step 9–10 at least twice to wash the cells. This step is critical to obtain a high purity of isolated cells.
12. Resuspend the bead-bound cells in 200 µL pre-heated (37° C) Buffer 3.
13. Add 4 µL reconstituted Release Buffer (DNase I).

14. Incubate for 15 min at room temperature with gentle tilting and rotation.
15. Pipet thoroughly with a 100–200 µL pipette at least 5–10 times to maximize cell release (avoid foaming).

Note: Before transferring the released cells to a new tube in the next step, pre-coat the tubes for 5 min with Buffer 3.

16. Place in a magnet for 2 min and transfer the supernatant with released cells into a pre-coated tube.
17. Resuspend the bead fraction in 200 µL Buffer 3 and repeat steps 15–16 once to collect residual cells.

Table 1: Volumes for indirect cell isolation.

Step	Step description	Volumes per 1×10^7 MNC	Volumes per 2×10^8 MNC
	Recommended tube size	5–7 mL	50 mL
	Recommended magnet	DynaMag™-5	DynaMag™-50
1	Primary mouse IgG antibody	~10 µg	~200 µg
1	Cell volume (MNC/blood)	1 mL	20 mL
3*	Wash cells (Buffer 2)	~2 mL	~40 mL
4	Resuspend cells	1 mL	20 mL
5**(*)	Add Dynabeads® magnetic beads	25 µL	500 µL
7*	*Optional:* Increase volume (Buffer 2)	~1 mL	~ 20 mL
10–11*	Wash cells (Buffer 1)	3 × ~1 mL	3 × 20 mL
12	Resuspend cells (Buffer 3)	200 µL	4 mL
13	Release cells (DNase I)	4 µL	80 µL
17	Collect residual cells (Buffer 3)	200 µL	4 mL

* Adjust the Buffer volumes to fit to the tube you are using.
** If the target cell population is high (e.g. >2.5 × 10^6 target cells/mL), increase the amount of Dynabeads® (maximum double the amount).
*** When incubating, tilt and rotate 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®)

- See "General Guidelines" for recommendation of when to use the direct vs. indirect cell isolation technique.
- Use 0.1–0.5 µg of primary mouse IgG antibody per 25 µL (1×10^7) Dynabeads®.
- 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 reagent and volumes accordingly, as shown in Table 2.

1. Transfer 25 µL pre-washed and resuspended Dynabeads® magnetic beads to a tube.
2. Add ~0.3 ug antibodies (titrate the antibody amount for your use).
3. Incubate for ≥30 minutes at room temperature 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 Buffer 1.
6. Repeat steps 4–5 once to remove excess of antibodies.
7. Place the tube in the magnet for 1 min and discard the supernatant.
8. Add to the beads 1 mL cell sample (blood or 1×10^7 MNC) and resuspend.
9. Incubate for 20 min at 2°C to 8°C with gentle tilting and rotation.
10. *Optional:* Add 1 mL Buffer 2 to limit trapping of unbound cells.
11. Place the tube in a magnet for 2 min.
12. 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 Buffer 1, pipet 2–3 times (or vortex 2–3 sec) and place the tube in a magnet for 2 min.
14. Repeat steps 12–13 at least twice to wash the cells and obtain high purity.
15. Resuspend the bead-bound cells in 200 µL pre-heated (37° C) Buffer 3.
16. Add 4 µL reconstituted Release Buffer (DNase I).
17. Incubate for 15 min at room temperature with gentle tilting and rotation.
18. Pipet thoroughly with a 100–200 µL pipette at least 5–10 times to maximize cell release (avoid foaming).
19. Before transferring the released cells to a new tube in the next step, pre-coat the tubes for 5 min with Buffer 3.
20. Place in a magnet for 2 min and transfer the supernatant with released cells into a pre-coated tube.
21. Resuspend the bead fraction in 200 µL Buffer 3 and repeat steps 19–20 once.

Table 2: Volumes for for direct cell isolation.

Step	Step description	Volumes per 1×10^7 MNC	Volumes per 2×10^8 MNC
	Recommended tube size	5–7 mL	50 mL
	Recommended magnet	DynaMag™-5	DynaMag™-50
1	Dynabeads®	25 µL	500 µL
2	Primary mouse IgG antibody	~1 µg	~20 µg
5–6*	Wash Dynabeads® (Buffer 1)	~2 × 2 mL	~2 × 40 mL
8**(*)	Cell volume	1 mL	20 mL
10*	*Optional:* Increase volume (Buffer 2)	~1 mL	~ 20 mL
13–14*	Wash the cells (Buffer 1)	3 × ~1 mL	3 × 20 mL
15	Resuspend cells (Buffer 3)	200 µL	4 mL
16	Release cells (DNase I)	4 µL	80 µL
20	Collect cells (Buffer 3)	200 µL	4 mL

* Adjust the Buffer volumes to fit to the tube you are using.
** If the target cell population is high (e.g. >2.5 × 10^6 target cells/mL), increase the amount of Dynabeads® (maximum double the amount).
*** When incubating, tilt and rotate 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

The CELLection™ Pan Mouse IgG Dynabeads® are uniform, superparamagnetic polystyrene beads (4.5 µm diameter) coated with monoclonal human anti-mouse IgG antibodies via a DNA linker to provide a cleavable site for cell release. The antibody coated onto Dynabeads® recognizes all mouse IgG subclasses, is Fc-specific and does not cross-react with human, rat, rabbit, guinea pig, sheep, goat or hamster IgG. The source of the human monoclonal antibody is free of Human Immunodeficiency Virus (HIV), Hepatitis-B Virus (HBV) and Hepatitis-C Virus (HCV). The Releasing Buffer Component 1 contains freeze-dried DNase 1 and needs to be reconstituted in Releasing Buffer Component 2 prior to use.

Related Products

Product	Cat. no.
DynaMag™-5	12303D
DynaMag™-15	12301D
DynaMag™-50	12302D
Dynabeads® Pan Mouse IgG	11041
Dynabeads® FlowComp™ Flexi	11061D
HulaMixer® Sample Mixer	15920D

REF on labels is the symbol for catalog number.

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