invitrogen

Dynabeads™ HLA CLASS I and CLASS II

Catalog nos. 21001D, 21002D, 21003, 21004D

Store at 2˚C to 8˚C

Publication No. MAN0009978 **Rev.** C.0

Product contents

Cat. No.	Volume	No. Isolations
Dynabeads™ HLA CLASS I		
21001D	2 mL	20
21002D	5 mL	50
Dynabeads™ HLA CLASS II		
21003	2 mL	20
21004D	5 mL	50

Dynabeads™ HLA Class I contains 1.4×10^8 beads/mL, and Dynabeads™ HLA Class II contains 2.8×10^8 beads/mL. The Dynabeads™ magnetic beads are suspended in phosphate buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) and 0.02% sodium azide.

Caution: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.

Product description

Dynabeads™ HLA Class I and Dynabeads™ HLA Class II are uniform, superparamagnetic polystyrene beads (4.5 µm diameter). Dynabeads™ HLA Class I is coated with a primary mouse anti-human monoclonal antibody specific for the CD8 membrane antigen on human cells. Dynabeads™ HLA Class II is coated with a primary mouse anti-human monoclonal antibody specific for the HLA Class II membrane antigen on human cells.

Dynabeads™ HLA Class I is designed for rapid isolation of $CD8^+$T lymphocytes directly from peripheral blood. The isolated cells can be used directly in microcytotoxic HLA Class I (A, B, C) assays.

Dynabeads™ HLA Class II is designed for rapid isolation HLA Class II^+ cells (mostly B lymphocytes) directly from peripheral blood. The isolated cells can be used directly in microcytotoxic HLA Class II (DR, DQ, and DP) assays.

The immunomagnetic cell isolation technique using the Dynabeads™ HLA Class I or the Dynabeads™ HLA Class II product enables a rapid and specific isolation of target cells directly from whole blood in only 15 min. The target cells are bound to the beads after a short incubation, and the bead-bound cells are isolated and washed using a magnet.

CE IVD For *in vitro diagnostic* use.

Dynabeads™ HLA Class I and Dynabeads™ HLA Class II yield purity up to 99% and viability up to 95% of the isolated cells.

The method is simple, rapid, and specific. When bound to the cell surface, the Dynabeads™ magnetic beads do not interfere with the binding of HLA-typing antibodies and complement to the cells.

Note: Viability of Dynabeads™ HLA Class II isolated cells after microcytotoxic incubation is typically lower than its Dynabeads™ HLA Class I counterpart.

Required materials

- DynaMag™ Magnet (Go to **thermofisher.com/magnets** for recommendations).
- Mixer allowing tilting and rotation of tubes.
- Blood collecting tubes containing anti-coagulant (e.g. acid citrate-dextrose [ACD], heparin).
- 5 mL pipettes.
- Container for blood and disposal.
- Cooling device.
- See Table 1 for recommended buffers and solutions.

General guidelines

- These products are for *in vitro diagnostic* use.
- Because mammalian cells are used in this procedure, follow appropriate laboratory techniques.
- Handle all samples as if they are capable of transmitting disease.
- Wear gloves and appropriate protection at all times.
- **Use a mixer that provides tilting and rotation of the tubes to ensure that Dynabeads™ magnetic beads do not settle in the tube.**
- Avoid air bubbles (foaming) during pipetting.
- Carefully follow the recommended pipetting volumes and incubation times.
- **Keep all buffers cold.**
- Cells should be isolated within 24 h to achieve the highest yield and viability. However, blood up to 3-days-old can be used. Older samples may yield lower cell viability.
- Blood samples from uremic and leukemic patients should be processed within 24 h after collection.

Table 1: Recommended buffers and solutions

PBS, pH 7.4, with sodium citrate*	Add 0.6% v/v sodium citrate.
PBS, pH 7.4, with EDTA*	Add 8% v/v EDTA.
Tris – balanced salt solution (TBSS), pH 7.4*	Add 2% heat inactivated fetal calf serum (FCS), or 2% heat inactivated human serum (HS) before use.
Quenching solution	India Ink diluted to 2.5% v/v in PBS.
Blocking solution	Add 8 g Na_2EDTA to 90 mL PBS, mix well, adjust pH to 7.4 with NaOH and then adjust the volume to 100 mL. The quenching and blocking solutions may be mixed and added to the well simultaneously.
AO/EB – stock solution	15 mg acridine orange (AO). 50 mg ethidium bromide (EB). Dissolve in 1 mL 95% ethanol. Add 49 mL PBS. Mix well. Divide into 1-mL aliquots and freeze.
AO/EB - working solution	Thaw 1 mL of stock solution, and dilute 1:10 in PBS, pH 7.4. Mix well. Store in an amber bottle at 2°C to 8°C for up to one month.
AO - stock solution	15 mg AO dissolved in 1 mL 95% ethanol. Add 49 mL PBS. Mix well. Divide into 1-mL aliquots and freeze.
AO - working solution	Thaw 1 mL of AO stock solution and dilute 1:10–1:20 in PBS, pH 7.4. Mix well. Store in amber bottle at 2°C to 8°C for up to one month.
EB - stock solution	50 mg EB dissolved in 1 mL 95% ethanol. Add 49 mL PBS. Mix well. Divide into 1-mL aliquots and freeze.
EB - working solution	Thaw 1 mL of EB stock solution and dilute 1:10–1:20 in PBS, pH 7.4. Mix well. Store in amber bottle at 2°C to 8°C for up to one month.
5-Carboxyfluorescein diacetate (CFDA)	Stock solution (Sigma-Aldrich, Cat.no. C4916). 10 mg of CFDA diluted in 1 mL acetone. Store at –70°C (solution will not freeze).

***Note:** Other buffers may be used for resuspension of cells, but they should be supplemented with Ca^{2+}, Mg^{2+}, and 2% FCS or 2% HS. Use analytical grade reagents.

Protocol

Prepare sample

In some patients, the number of HLA Class I and II cells are low (e.g., hematological disorders, leukemic, uremic), or the serum may contain high quantities of soluble HLA Class I and II molecules, interfering with the cell isolation capacity of the beads. To give a satisfactory yield for HLA Class I and HLA Class II cells for typing, use the following procedure prior to "Isolate cells" for samples known to give low lymphocyte yields in conventional cell isolation procedures.

For buffy coat isolation:

1. Centrifuge 10 mL of blood at 800–1000 × *g* for 5 min without brakes.
2. Discard plasma and transfer the buffy coat, and the very upper layer of erythrocytes to a clean 10 mL tube.
3. Add 5 mL cold PBS with 0.6% sodium citrate, pH 7.4 to the buffy coat. Mix gently.
4. Continue to "Isolate cells".

For whole blood isolation:

1. Collect 5 mL blood (ACD) in a standard blood collecting tube, and cool **at 2°C to 8°C** to prevent attachment of phagocytic cells to the beads.
2. Add 5 mL cold PBS with 0.6% sodium citrate to each sample.
3. Continue to "Isolate cells".

Isolate cells

1. Resuspend the Dynabeads™ magnetic beads (i.e., vortex for >30 sec, or tilt and rotate for 5 min). **Note:** keep beads in the rotator until use. **For better performance, wash beads with PBS/citrate buffer** (mix 100 µL of beads with 4–5 mL PBS-citrate solution, place tube in the magnet for 2 min, remove supernatant, resuspend beads in 100 µL of PBS-citrate).
2. Add 100 µL of beads to the blood sample.
3. Mix for **5 min at 2°C to 8°C** by gentle tilting and rotating.
   Note: Do not use end-over-end rotation, as this may damage target cells and increase background in the microcytotoxic assay.

4. Add 5 mL **cold** PBS with 0.6% sodium citrate, pH 7.4, pipet up and down a few times, before placing the tube in the magnet for 2 min.
5. Discard the supernatant without disturbing the pellet containing the bead-bound cells.
6. Remove the tube from the magnet and resuspend the bead-bound cells in 5 mL **cold PBS**, pH 7.4, with 0.6% sodium citrate.
7. Apply to the magnet for 2 min to collect the bead-bound cells, and discard the supernatant while the tube is still in the magnet.
8. Repeat step 6–7 four times, except use 5 mL **cold** PBS, pH 7.4, **without** sodium citrate for each wash.
9. Resuspend the bead-bound cells in 0.4 mL TBSS, pH 7.4, containing Ca^{2+} and Mg^{2+} with 2% heat inactivated FCS or 2% heat inactivated HS, or equivalent.
   Note: Other buffers may be used for resuspension of cells, but they should be supplemented with Ca^{2+}, Mg^{2+}, and 2% FCS or 2% HS. Use analytical grade reagents. Use bead-bound cells immediately (cells can be kept at 2°C to 8°C for up to 24 hours if necessary).

Stain isolated cells

For staining of cells isolated with Dynabeads™ HLA Class I or Dynabeads™ HLA Class II used in microcytotoxic assays, we recommend staining with fluorescent dyes (e.g., acridine orange (AO), and ethidium bromide (EB)). Assessment of viable (green) and dead (red) cells can be made with a fluorescence microscope (excitation filter 450/490 nm). The cytotoxic reactions will proceed after addition of staining solution. These reactions can be stopped by adding 8% EDTA dissolved in PBS to the well. The EDTA will maintain the fluorescence of the tray overnight.

Note: Fluorescent stains require the trays to be incubated in the dark at room temperature (20°C to 22°C). The stain should be kept out of direct light and stored in the dark.

Note: A two-step staining procedure can be used with the inverted fluorescence microscope. The two-step staining protocol, without the addition of India Ink, must be used with the upright fluorescence microscope.

Inverted fluorescence microscope staining

One-step staining protocol

1. Bead-bound cells are dispersed into the tray wells.
2. Incubate with the sera and complement, and add 1 µL of AO or EB working solution into each well.
   or
   Dispense the staining solution into the wells together with the complement: add 2 mL rabbit complement (or the amount of complement indicated by the tray manufacturer), and 40 µL of AO or EB stock solution.
3. Incubate for 30 min. Read the tray within 1 hour after adding the staining solution, when EDTA is not used for blocking the staining reaction.

Note: When using Terasaki trays it can be difficult to distinguish the negative (green) cells from the background. The background staining can be quenched by adding 5 µL appropriately diluted India Ink or hemoglobin to each well.

Two-step staining protocol (AO)

AO is used as the negative cell stain.

1. Add 2 drops of AO working solution to the test tube, resuspend, and incubate for 30 sec at room temperature.
2. Add 5 mL PBS, resuspend, and apply to the magnet for 1 min. Discard the supernatant.
3. Resuspend the cells in TBSS or equivalent with 2% heat inactivated FCS or 2% heat inactivated HS.
4. Add complement and 1 µL EB working solution to each well.

Note: The background will be light orange/brown, thus the addition of India Ink is not necessary to provide sufficient cell-to-background fluorescent contrast. If the trays are to be read the next day, reactions can be refrigerated after adding 5 µL of 8.0% EDTA in PBS to each well. If further reduction of background is desired, add 5 µL of India Ink diluted to 2.5% v/v with PBS containing 8.0% EDTA.

Two-step staining protocol (CFDA)

CFDA is used as the negative cell stain.

1. Add 0.2 mL CFDA working solution to the test tube, resuspend, cover the test tube, and incubate for 15 min at 37°C.
2. Add 5 mL PBS, resuspend, and apply to the magnet for 1 min. Discard the supernatant.
3. Repeat step 2 once, and resuspend cells in TBSS or equivalent with 2% heat inactivated FCS or 2% heat inactivated HS.
4. Add complement and 1 µL EB working solution to each well.

See **"Note"** for "Two-step staining protocol (AO)" for further background staining info.

The CFDA and EB two-step staining technique provides the greatest contrast between negative and positive cells, and the background. This is especially true if India Ink is added to the wells, as the Dynabeads™ magnetic beads will be obscured.

Upright fluorescence microscope staining

- The "One-step staining protocol" under inverted fluorescence scopes is not applicable for upright fluorescence, since the AO background is too intense, thus the cell visibility is obscured.
- The "Two-step staining protocols" under inverted fluorescence scopes are applicable to the upright fluorescence microscopes. The addition of India Ink is not applicable, since the cells will not be visible.

Related products

Product	Cat. No.
DynaMag™-5 magnet	12303D
DynaMag™-15 magnet	12301D
MPC™-1 magnet	12001D

Explanation of symbols

REF	Catalog number
Σ	Contains sufficient for *n* tests
Read SDS	Read safety data sheet

Open vials have the same shelf life as printed on the lot-specific label, as long as stored at 2°C to 8°C, and contamination is avoided.

References are available on the product specific page on **thermofisher.com**.

Limited product warranty

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Valid Edition of the Instructions for use

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