

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
 - 1.3 Recommended antibody dilution
 - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
 - 2.1 Extracellular staining
 - 2.2 Intracellular staining of cells in suspension
3. Examples of immunofluorescent staining and Western blotting with Anti-Dectin-2 antibodies
4. References

1. Description

Components	1 mL Anti-Dectin-2 antibodies, mouse: monoclonal Anti-Dectin-2 antibodies conjugated to fluorescein isothiocyanate (FITC). The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
Clone	KVa7-6E7 (isotype: rat IgG2a).
Capacity	100 tests or up to 10 ⁹ total cells.
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

Mouse dendritic cell-associated C-type lectin 2 (Dectin-2) is a type II membrane protein and contains a single carbohydrate recognition domain. Like other C-type lectins, mouse Dectin-2 has conserved motifs for the Ca²⁺-dependent recognition of mannose. Dectin-2 is located in the natural killer gene complex of chromosome 6. Genomic analyses show that beside a full length Dectin-2 transcript two truncated isoforms are produced by alternative splicing, encoding transmembrane proteins of 168–209 amino acids.^{1,2} The expression of Dectin-2 can be induced by zymosan and thioglycollate on mouse monocytes and macrophages.³ Dectin-2 plays a physiological role in antigen presentation and antigen targeting via Dectin-2 can induce a CD8⁺ T cell response.⁴

1.2 Applications

- Identification and enumeration of Dectin-2⁺ cells by flow cytometry or fluorescence microscopy.
- Detection of mouse Dectin-2 by immunoblot analysis.

1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

Anti-Dectin-2 antibody	FITC	pure
Flow cytometry		
- In general	1:11	
- Formaldehyde-fixed cells	1:11	
- Intracellular staining	1:11	
Western blotting		0.5–5 µg/mL

1.4 Reagent requirements

- Buffer: Prepare a solution containing Ca²⁺ and Mg²⁺, pH 7.2, e.g., Hanks' Balanced Salt Solution (HBSS): 138 mM NaCl, 5.3 mM KCl, 0.2 mM Na₂HPO₄, 0.4 mM KH₂PO₄, 1.3 mM CaCl₂, 0.8 mM MgSO₄, 4.2 mM NaHCO₃. Add 0.5% bovine serum albumin (BSA). Keep buffer cold (2–8 °C).
- Phosphate-buffered saline (PBS), pH 7.2. Keep PBS cold (2–8 °C).
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.
- (Optional) CD11c-PE (# 130-091-830) or CD11b-APC (# 130-091-241). For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.
- (Optional) Inside Stain Kit (# 130-090-477) for fixation and permeabilization of cells.
- (Optional) Anti-rat horseradish peroxidase conjugates for Western blotting.

2. General protocol for immunofluorescent staining

2.1 Extracellular staining

▲ Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Anti-Dectin-2 antibody.

5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
 - ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

2.2 Intracellular staining of cells in suspension

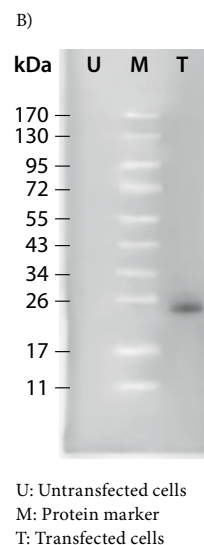
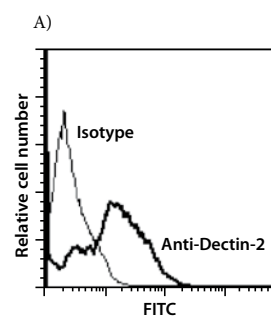
▲ It is recommended to stain 10^6 cells per sample. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Wash up to 10^6 cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. (Optional) Stain cell surface antigens that are sensitive to fixation according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 cells in 250 μ L of PBS.
4. Add 250 μ L of Inside Fix (Inside Stain Kit). Mix well and incubate for 20 minutes at room temperature.
5. Centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
6. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
 - ▲ **Note:** Fixed cells may be stored at 2–8 °C for up to 1 week.
7. (Optional) Stain cell surface antigens that are sensitive to permeabilization with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cells in 100 μ L of Inside Perm (Inside Stain Kit). Mix well and incubate for 20 minutes at room temperature.
9. Add 10 μ L of the Anti-Dectin-2 antibody.
10. (Optional) Add additional staining antibodies to the solution, for example, for the staining of cell surface antigens internalized upon cell activation.
 - ▲ **Note:** For efficient permeabilization upon intracellular staining the volume of Inside Perm should be at least 5× the volume of staining antibodies.
11. Mix well and incubate for 10 minutes in the dark at room temperature.
12. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
13. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric acquisition.
 - ▲ **Note:** Samples may be stored at 2–8 °C in the dark for up to 24 hours.
 - ▲ **Note:** Do not use propidium iodide (PI) or 7-AAD staining.

3. Examples of immunofluorescent staining and Western blotting with Anti-Dectin-2 antibodies

Bone marrow from GM-CSF treated BALB/c mice was intracellularly stained with Anti-Dectin-2-FITC (# 130-094-480) or rat IgG2a isotype antibodies conjugated to FITC (A) and analyzed by flow cytometry. Cell debris were excluded from the analysis based on scatter signals.

For Western blotting, 1.3×10^6 human embryonic kidney (HEK) 293 cells were used. The cells were either untransfected or transfected with full-length mouse Dectin-2. Lysates were size fractionated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membrane. Dectin-2 expression was detected with 5 μ g/mL Anti-Dectin-2 pure followed by anti-rat horseradish peroxidase conjugate and enhanced chemiluminescence (ECL) substrate (B).



4. References

1. Fernandes, M.J. *et al.* (1999) Characterization of a Novel Receptor That Maps Near the Natural Killer Gene Complex: Demonstration of Carbohydrate Binding and Expression in Hematopoietic Cells. *Cancer Res.* 59: 2709–2717.
2. Ariizumi, K. *et al.* (2000) Cloning of a Second Dendritic Cell-associated C-type Lectin (Dectin-2) and Its Alternatively Spliced Isoforms. *J. Biol. Chem.* 275: 11957–11963.
3. Taylor, P.R. *et al.* (2005) Dectin-2 is predominantly myeloid restricted and exhibits unique activation-dependent expression on maturing inflammatory monocytes elicited in vivo. *Eur. J. Immunol.* 35: 2163–2174.
4. Carter, R.W. *et al.* (2006) Induction of CD8⁺ T cell responses through targeting of antigen to Dectin-2. *Cell Immunol.* 239: 87–91.

All protocols and data sheets are available at www.miltenyibiotec.com.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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