

Loading of CD1d monomer with lipids

Products	hCD1d/unloaded, Cat. No. XD08001M 20/100/200/1000/2000 mCD1d/unloaded, Cat. No. YD08001M 20/100/200/1000/2000
Recommended use	This protocol describes how to load CD1d monomer with glycolipids of interest and if needed how to use the resulting CD1d/lipid monomers to create Dextramer [®] and dCODE Dextramer [®] reagents for immune monitoring and single cell analysis of NKT cells.
Material provided	CD1d/unloaded (human or mouse, biotinylated, Mw 50.000)
Material not provided	Lipid of interest (e.g., α -Galactosyl Ceramide, or another glycolipid) DMSO 0.5 % Tween-20 in phosphate-buffered saline (PBS) Heating block or water bath (80 °C capability) Incubator (30 °C, without shaking) Microcentrifuge tubes (low protein-binding recommended) Analytical balance (for weighing lipid stocks, if needed) U-Load Dextramer [®] (including dilution buffer - to make CD1d Dextramer [®] , if needed) U-Load dCODE Dextramer [®] (including dilution buffer - to make CD1d dCODE Dextramer [®] , if needed)
Procedure	<p>Lipid loading of CD1d</p> <p>1. <u>Preparation of Reagents:</u> <i>Prepare the lipid stock solution</i> <ul style="list-style-type: none"> • Weigh the desired amount of lipid, resuspend in a suitable solvent (e.g., DMSO or PBS with 0,05 % Tween-20) to make a 1mM stock solution. • Sonicate briefly or vortex to ensure complete dissolution. • Aliquot and store at -80 °C if not using the solution immediately. <i>Prepare CD1d protein solution</i> <ul style="list-style-type: none"> • Thaw CD1d at 2-8 °C or on ice. • Dilute CD1d in PBS to a working concentration of 1 mg/mL (20 μM). • Keep the solution at room temperature (RT). </p> <p>2. <u>Pre-Heating and Lipid Solubilization:</u> <i>Mix lipid with PBS/0.5% Tween-20</i> <ul style="list-style-type: none"> • Dilute lipid stock solution (1 mM) with PBS containing 0.5 % Tween-20 in a fresh microcentrifuge tube to a final concentration of 120 μM. <i>Heat lipid solution</i> <ul style="list-style-type: none"> • Place the tube in a preheated 80 °C heating block for 2-5 minutes to improve solubilization. • Vortex gently if necessary to ensure lipid is fully dissolved. • Allow the solution to cool to 30 °C. (For temperature-sensitive lipids, skip heating or use lower temperatures as needed.) </p> <p>3. <u>Lipid Loading into CD1d</u> <i>Combine lipid and CD1d</i> </p>

- Add equal volumes of solubilized lipid and CD1d protein solutions in a microcentrifuge tube (lipid-to-CD1d molar ratio should be 6:1).
- Mix gently by pipetting up and down, without making bubbles (do not vortex).

Incubate for lipid loading

- Incubate the tube at 30 °C overnight.
- Store the lipid loaded CD1d monomer at 4 °C or at -80 °C for long-term storage.

4. Create CD1d Dextramer® or CD1d dCODE Dextramer® reagents (if needed)

CD1d Dextramer®

- To load the CD1d/lipid monomer onto U-Load Dextramer®, mix the reagents in Table A in a 1.5 mL tube (see Procedural notes):

Table A

Reagents	10 tests	20 tests	50 tests
U-Load Dextramer®	20 µL	40 µL	100 µL
CD1d/lipid monomer	7 µL	14 µL	35 µL
<i>Incubate for 30 min at RT in the dark</i>			
U-Load Dextramer® Dilution buffer	73 µL	146 µL	365 µL
Total volume	100 µL	200 µL	500 µL

- Store the fluorescent CD1d/lipid U-Load Dextramer® reagent at 2-8 °C in the dark until use.

CD1d dCODE Dextramer®

- To load the CD1d/lipid monomer onto the U-Load dCODE Dextramer®, mix the reagents in Table B in a 1,5 mL tube:

Table B

Reagents	10 tests	20 tests	50 tests
U-Load dCODE Dextramer®	12 µL	23.5 µL	59.5 µL
CD1d/lipid monomer	7 µL	14 µL	34.5 µL
<i>Incubate for 30 min at RT in the dark</i>			
U-Load dCODE Dextramer® Dilution buffer	1 µL	2.5 µL	6 µL
Total volume	20 µL	40 µL	100 µL

- Store the fluorescent CD1d/lipid U-Load dCODE Dextramer® reagent at 2-8 °C in the dark until use.

5. Staining procedures

CD1d/lipid Dextramer®

- To analyse NKT cells in blood by flow cytometry with CD1d/lipid U-Load Dextramer®, see the MHC Dextramer® Staining Protocol at immudex.com/resources/protocols/.

CD1d/lipid dCODE Dextramer®

- For staining procedures and sequencing workflows please find protocols for the various types of dCODE[®] products (HiT, 10x and RiO) at our website: immudex.com/resources/protocols/.

Procedural Notes

N.B.: U-Load Dextramer[®] with APC fluorophores require different volumes of reagents!

- To assemble the CD1d/lipid monomer with U-Load Dextramer[®] APC, mix the reagents in Table C in a 1.5 mL tube:

Table C

Reagents	10 tests	20 tests	50 tests
U-Load Dextramer [®] APC	20 µL	40 µL	100 µL
CD1d/lipid monomer	4.5 µL	9 µL	23 µL
<i>Incubate for 30 min at RT in the dark</i>			
U-Load Dextramer [®] Dilution buffer	75.5 µL	151 µL	377 µL
Total volume	100 µL	200 µL	500 µL

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Technical support

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