

## Loading of CD1d Dextramer<sup>®</sup> with lipids

<b>Intended use</b>	This procedure has been successfully used for loading $\alpha$ -Galactosyl Ceramide lipid into human and mouse CD1d/unloaded Dextramer <sup>®</sup> . In this protocol the CD1d monomer is mixed with approximately 15 molar excess of lipid during loading, but optimal conditions and molar stoichiometry can vary between lipids.
<b>Optimized for</b>	Cat. No. XD8001, YD8001 CD1d Dextramer <sup>®</sup>
<b>Materials Provided</b>	CD1d/unloaded Dextramer <sup>®</sup> - labeled with PE or APC
<b>Materials Required (not provided)</b>	Lipid (e.g. $\alpha$ -GalCer) dissolved in DMSO at 1 mg/ml (1.2 mM) 0.5 % tween-20 in PBS*
<b>Procedure</b>	<p><u>Procedure for lipid loading of CD1d Dextramer<sup>®</sup> sufficient for 10 tests:</u></p> <ol style="list-style-type: none"><li>1. Heat the lipid stock solution to 80 °C for 1 min to completely dissolve it. Dilute an aliquot of lipid in 0.5 % Tween-20, PBS*, to a final concentration of 0.2 mg/ml and heat it to 80 °C for 1 min to maintain the lipid completely dissolved. Cool the lipid to 30 °C.</li><li>2. Add 5 <math>\mu</math>l of the lipid to 100 <math>\mu</math>l of CD1d/unloaded Dextramer<sup>®</sup> tempered to 30 °C.</li><li>3. Incubate at 30 °C overnight in the dark.</li><li>4. Store the lipid loaded CD1d Dextramer<sup>®</sup> at 4 °C in the dark until use.</li></ol>
<b>Procedural Notes</b>	<p>Always keep MHC Dextramer<sup>®</sup> stored at 2-8°C in the dark – the brown plastic vial does not sufficiently protect the reagents against light.</p> <p>* PBS: 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.47 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4</p>
<b>Technical Support</b>	For additional Tips & Tricks, FAQs, and protocols, please visit <a href="https://www.immudex.com/resources/">https://www.immudex.com/resources/</a> or contact our support team at <a href="mailto:customer@immudex.com">customer@immudex.com</a> Telephone: +45 3110 9292 (Denmark)
<b>Staining protocol</b>	MHC Dextramer <sup>®</sup> Staining Protocol: ( <a href="https://www.immudex.com/resources/protocols/#CD1d">https://www.immudex.com/resources/protocols/#CD1d</a> )