

**Streptavidin-R-PE:** In order to identify antigen-specific CD4<sup>+</sup> T lymphocytes, fluorochrome-labeled Class II tetramers are required. ProM2<sup>®</sup> human Class II MHC Monomer reagents can be made into Class II tetramers when combined with Streptavidin fluorochrome conjugates. Streptavidin has four biotin-binding sites, enabling biotinylated ProM2<sup>®</sup> human Class II MHC Monomer reagents to form Class II tetramers. CD4<sup>+</sup> T cells stained with Class II tetramers can be analyzed by flow cytometry and the frequency of antigen-specific T cells determined.

**For Research Use Only. Not for use in therapeutic or diagnostic procedures.**

**Vial specification:** One vial contains sufficient reagent to conjugate 35 µg ProM2<sup>®</sup> human Class II MHC Monomer.  
Two vials contains sufficient reagent to conjugate 100 µg ProM2<sup>®</sup> human Class II MHC Monomer.

**Test volume:** 65 µl Streptavidin-R-PE / 35 µg ProM2<sup>®</sup> Monomer  
180 µl Streptavidin-R-PE / 100 µg ProM2<sup>®</sup> Monomer

**Concentration/  
Formulation:** Streptavidin-R-PE is supplied at a concentration of 0.8 mg/ml in PBS stabilized with 3% BSA and 0.05% sodium azide.

**Storage Condition:** 4°C. Protect from light. **Do not freeze.**

**Shelf Life:** 6 months if stored as instructed above.

**Fluorochrome:** R-phycoerythrin (R-PE): excites at 480, 565 nm; emits at 578 nm.

**Hazards:** This reagent is formulated in 0.05% sodium azide. Under acid conditions the toxic compound hydrazoic acid may be released. Compounds containing sodium azide should be flushed with running water while being discarded.

### Quality Control Assay Results

**Appearance:** Clear, pink solution

**Protein Characterization:** Passed

**Released by:**  
(Date as per product label above)

### **Class II Tetramer Production Protocol:**

**Additional materials required:** ProM2<sup>®</sup> human Class II MHC Monomer, PBS containing 0.025% sodium azide.

1. Spin Streptavidin-R-PE in a chilled microcentrifuge at 14,000 ×g for 3 minutes. This will remove protein aggregates that contribute to non-specific staining. Maintain reagents on ice, shielded from light, until required. Do not aspirate any part of the pelleted aggregates when taking test volumes for conjugation.

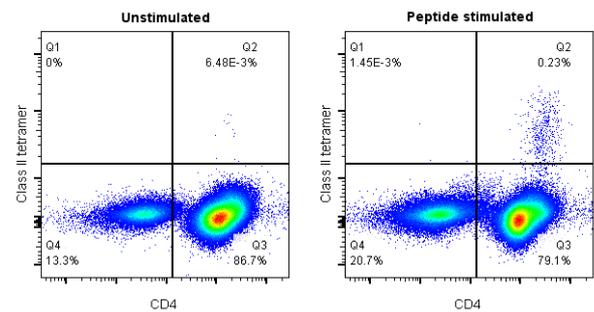
2. *To conjugate to 35 µg ProM2<sup>®</sup> human Class II MHC Monomer:*

Add 13 µl of 0.8 mg/ml Streptavidin-R-PE to 35 µg ProM2<sup>®</sup> human Class II MHC Monomer, mix gently and incubate at 4°C for 15 minutes. Repeat the addition of Streptavidin-R-PE four times with a 15 minute gap between each addition. Make up to a final volume of 400 µl with PBS/0.025% sodium azide.

*To conjugate to 100 µg ProM2<sup>®</sup> human Class II MHC Monomer:*

Add 36 µl of 0.8 mg/ml Streptavidin-R-PE to 100 µg ProM2<sup>®</sup> human Class II MHC Monomer, mix gently and incubate at 4°C for 15 minutes. Repeat the addition of Streptavidin-R-PE four times with a 15 minute gap between each addition. Make up to a final volume of 1.15 ml with PBS/0.025% sodium azide.

Store Class II tetramers at 4°C, protected from light. **Do not freeze.**



1 × 10<sup>6</sup> cells were incubated with 1 test size R-PE-labeled Class II tetramer at 37°C for 2 hours. Non-specific staining was eliminated from the plot by gating on CD19<sup>-</sup> cells before plotting CD4 vs Class II tetramer.