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PRODUCT SHEET
Streptavidin-R-PE:
- KM2X-

Streptavidin-R-PE:	In order to identify antigen-specific $CD4^+$ T lymphocytes, fluorochrome-labeled Class II tetramers are required. ProM2® 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® human Class II MHC Monomer reagents to form Class II tetramers. $CD4^+$ 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® human Class II MHC Monomer. Two vials contains sufficient reagent to conjugate 100 µg ProM2® human Class II MHC Monomer.
Test volume:	65 µl Streptavidin-R-PE / 35 µg ProM2® Monomer 180 µl Streptavidin-R-PE / 100 µg ProM2® 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)

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Class II Tetramer Production Protocol:

Additional materials required: ProM2® 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.

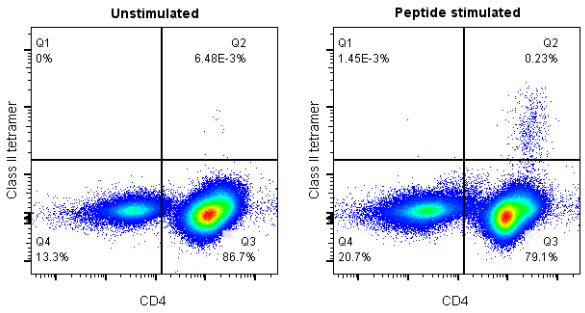
2. *To conjugate to 35 µg ProM2® human Class II MHC Monomer:*

   Add 13 µl of 0.8 mg/ml Streptavidin-R-PE to 35 µg ProM2® 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® human Class II MHC Monomer:

   Add 36 µl of 0.8 mg/ml Streptavidin-R-PE to 100 µg ProM2® 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 × 10^6 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 $CD19^-$ cells before plotting CD4 vs Class II tetramer.

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