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**PRODUCT SHEET**
**Streptavidin-APC:**
- KM4X-

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| **Streptavidin-APC:** | 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.<br>**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.<br>Three vials contains sufficient reagent to conjugate 100 µg ProM2® human Class II MHC Monomer. |
| **Conjugation volume:** | 115 µl Streptavidin-APC / 35 µg ProM2® Monomer<br>325 µl Streptavidin-APC / 100 µg ProM2® Monomer |
| **Concentration/ Formulation:** | Streptavidin-APC is supplied at a concentration of 0.09 mg/ml in PBS stabilized with 2% 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:** | Allophycocyanin (APC): excites at 650 nm; emits at 660 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, pale blue 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-APC 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.

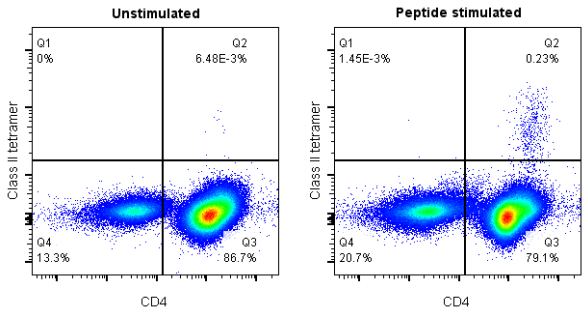
2. *To conjugate to 35 µg ProM2® human Class II MHC Monomer:*

   Add 23 µl of 0.09 mg/ml Streptavidin-APC to 35 µg ProM2® human Class II MHC Monomer, mix gently and incubate at 4˚C for 15 minutes. Repeat the addition of Streptavidin-APC 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 65 µl of 0.09 mg/ml Streptavidin-APC to 100 µg ProM2® human Class II MHC Monomer, mix gently and incubate at 4˚C for 15 minutes. Repeat the addition of Streptavidin-APC 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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