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PRODUCT SHEET
Hu MR1 Tetramer R-PE labeled
6-FP Loaded:
- YH002-2X

Not for US / AUS

MHC class I related protein (MR1) molecules are non-polymorphic, non-classical major histocompatibility complex (MHC) molecules that are highly conserved across many mammalian species. The ligand binding groove of the MR1 molecule has been shown to bind small molecules derived from microbial riboflavin biosynthetic pathways and present these to mucosal associated invariant T cells (MAIT cells). MAIT cells are a subset of unconventional T cells thought to play a role in microbial and mucosal immune responses. Presentation of microbial antigens in the context of MR1 leads to activation of MAIT cells, secretion of cytokines and lysis of microbial-infected cells.

6-Formylpterin (6-FP) binds to MR1 but has been shown to not stimulate MAIT cell responses. As such it has been used as a reliable negative control when staining MAIT cells with MR1. 5-amino-6-ribitylaminouracil (5-A-RU) was later identified as the specific intermediate of riboflavin synthesis which combines non-enzymatically with methylglyoxal or glyoxal, to form MR1 ligands 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU) and 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU), respectively. 5-OP-RU and 5-OE-RU elicit a strong response from MAIT cells and have become the favored ligands for MR1 loading and MAIT cell detection.

Human MR1 Tetramer 6-FP loaded:	ProImmune's fluorescent-labeled human MR1 tetramers loaded with 6-FP are used as a negative control and will not bind MAIT cells. Use of this negative control reagent in conjunction with an MR1 tetramer loaded with a MAIT cell stimulating ligand (e.g. 5-OP-RU) will allow low frequency positive populations to be accurately quantified. **For Research Use Only. Not for use in therapeutic or diagnostic procedures**.
Test Volume:	10 µl / test.
Test Specification:	One test contains sufficient reagent to stain approximately 1×10^6 cells. We recommend that the user titrate the reagent to determine the optimum amount to use in their specific application.
Concentration/ Formulation:	The MR1 Tetramer concentration is approximately 0.1 mg/ml in PBS, stabilized with 0.5% BSA and 0.025% 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 and emits at 578 nm (FL-2)
Hazards:	This reagent is formulated in 0.025% 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 pink solution

Protein Characterization: Passed

Released by:
(Date as per product label above)

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Cellular Staining Protocol

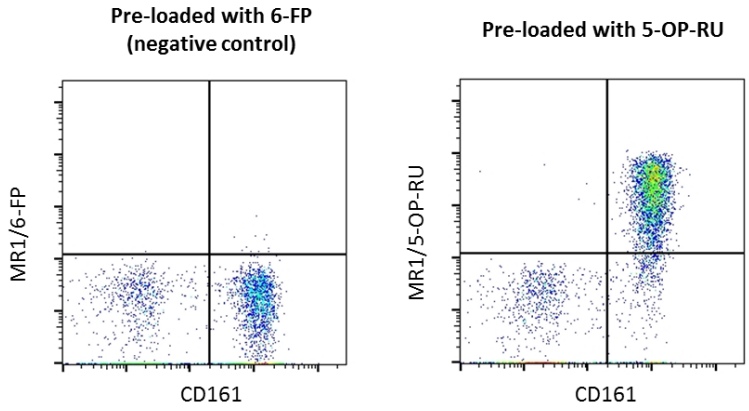
Additional materials required: Wash Buffer (0.1% sodium azide, 0.1% BSA in PBS), Fix Solution (1% fetal calf serum, 1% formaldehyde in PBS), anti-CD3 antibody, anti-CD19 antibody, anti-CD161 antibody and anti-human TCR Vα7.2 antibody.

1. **Centrifuge MR1 tetramers in a chilled microcentrifuge at 14,000 ×*g* for 5 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 tests for staining.
2. **Allocate 1-2 × 10^6 lymphoid cells (PBMCs) per staining condition.** Allocate only 2-5 × 10^5 cells per staining condition when using T cell clones or lines, due to the higher frequency of antigen-specific T cells.
3. **Wash the cells with 2 ml Wash Buffer and resuspend them in the residual volume (~50 µl).** Keep tubes chilled on ice for all subsequent steps, except where indicated.
4. **Add one test (10 µl) of fluorescent-labelled MR1 tetramer to the cells and mix well.**
5. **Incubate samples at 4 °C for 30 minutes in the dark.**
6. **Wash the cells with 2 ml Wash Buffer and resuspend them in the residual volume.**
7. **Add anti-CD3, anti-CD19, anti-CD161 and anti-human TCR Vα7.2 antibodies to the cells and mix well.** Use of the anti-CD19 antibody is recommended to exclude non-specific staining of B cells from your cytometry analysis. Use of anti-CD161 and anti-human TCR Vα7.2 antibodies may be used to definitively identify the MAIT cell population
8. **Incubate samples at 4 °C for 30 minutes in the dark.**
9. **Wash the cells twice with 2 ml Wash Buffer and resuspend thoroughly before adding 200 µl Fix Solution.** Store them in Fix Solution at 4 °C, in the dark, until analysis

Tetramer-positive cells are most conveniently viewed by gating first on live, CD19-negative lymphoid cells, followed by gating on CD3+/TCR Vα7.2+ cells. MR1-reactive MAIT cells can be analyzed on a two-color plot showing CD161 on the *x*-axis and Tetramer on the *y*-axis.

Protocol Optimization

For further tips on protocol optimization refer to http://www.proimmune.com/support-protocol-optimization or download the Pro5® MHC Pentamer Handbook which contains useful protocols and advice on how to achieve the best possible staining for your samples (https://www.proimmune.com/ecommerce/pdf_files/Pentamer-Handbook.pdf).



The figure on the left shows a cell sample stained with Negative Control Tetramer (Code YH002). The figure on the right shows the same cells stained with the 5-OP-RU loaded MR1 tetramer. A population of MAIT cells is clearly visible in the upper right quadrant. MAIT cells were identified by gating for CD19$^-$/CD3$^+$/TCR Vα7.2$^+$ before plotting CD161 vs MR1 tetramer.

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