

# Human TCR Tetramer-PE

## (HLA-A\*24:02 survivin-2B<sub>80-88</sub>-AYACNTSTL)

For Research Use Only. Not for use in diagnostic procedures.

### Background

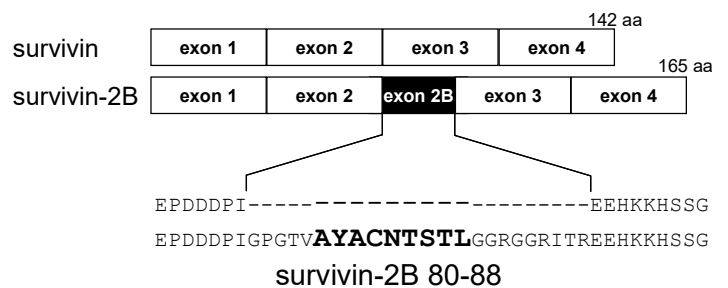
Tumor-associated antigens (TAAs) have been identified in patients with various cancers, and are potential targets for cancer immunotherapy. TAAs can be presented as peptides in the human leukocyte antigen (HLA) on the cell surface, which interact with the T cell receptors (TCR) present on antigen-specific T cells.

TCR-peptide/HLA interaction is the gold standard of immune responses as it induces antigen-specific cytotoxic T lymphocytes (CTL). Several immunotherapies have played pivotal roles in cancer treatment by targeting the TCR-peptide/HLA interactions. Studies on the interaction between individual TCRs and their specific peptide/HLA complexes can reveal the biological functions of T cells and may help in designing TCR-based immunotherapy strategies.

The TCR tetramer reagent comprises a soluble TCR complex conjugated with a fluorescent protein that recognizes HLA-A\*24:02 presenting a survivin-2B-derived epitope peptide (survivin-2B<sub>80-88</sub>). Thus, it can detect HLA-A\*24:02-restricted survivin-2B<sub>80-88</sub>-specific antigen-presenting cells by flow cytometry.

Survivin is a member of the inhibitor of apoptosis protein (IAP) family and is functionally involved in both inhibition of apoptosis and regulation of cell division. There are five different isoforms which include the wild-type survivin, survivin-2B, survivin-3B, survivin-ΔEx3, and survivin-2α. Survivin-2B originates from the insertion of an alternative exon 2B. Dr. Noriyuki Sato and his colleagues at Sapporo Medical University previously reported that survivin-2B was expressed abundantly in various types of tumor tissues and suitable as a target antigen for peptide-based cancer immunotherapy. They have identified an HLA-A24-restricted antigenic peptide, survivin-2B<sub>80-88</sub> (AYACNTSTL) derived from exon 2B-encoded region, recognized by CD8<sup>+</sup> cytotoxic T lymphocytes (CTL). This CTL epitope has high potency for CTL induction in various cancer patients, including those with breast cancer, colorectal cancer, gastric cancer and

oral cancer. A phase II clinical study of survivin-2B peptide vaccination is initiated for patients with advanced or recurrent pancreatic cancer in Japan.



### TCR Recognition

HLA-A\*24:02 presenting survivin-2B peptide (80–88 aa, AYACNTSTL)

### Reagents

500 μL liquid - 10 μL/test

The Tetramer is dissolved in an aqueous buffer containing 0.5 mM EDTA, 0.2% BSA, 10 mM Tris-HCl (pH 8.0), 200 mM NaCl, and 0.09% NaN<sub>3</sub>.

### Conjugates

Streptavidin-Phycoerythrin (SA-PE)

Excites at 486-580 nm

Emits at 586-590 nm

### Storage Conditions

Store at 2 to 8°C. Do not freeze. Minimize exposure to light. The expiration date is indicated on the vial label.

### Evidence of Deterioration

Any change in the physical appearance of this reagent may indicate deterioration and the reagent should not be used. The normal appearance is a clear, colorless to pink (SA-PE).

### Usage

This reagent is for use with standard flow cytometry methodologies.

## Statement of Warnings

1. This reagent contains 0.09% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipette by mouth and avoid contact of samples with skin and mucous membranes.
4. Minimize exposure of reagent to light during storage or incubation.
5. Avoid microbial contamination of reagent or erroneous results may occur.
6. Use Good Laboratory Practices (GLP) when handling this reagent.

## Materials Required But Not Supplied

- 12 x 75 mm polypropylene test tubes
- Transfer pipettes
- Pipettors and disposable pipette tips
- Vortex mixer
- Centrifuge capable of 150 x g or 400 x g
- Aspirator
- PBS
- Anti-HLA-A24 (Human) mAb-FITC, MBL, code no. K0209-4
- 7-AAD Viability Dye, Beckman Coulter, Inc., code no. A07704
- Clear Back (human FcR blocking reagent), MBL, code no. MTG-001

## Protocol

### 1) Peptide pulsed

1. Prepare cultured cells in medium without fetal bovine serum (FBS). If your growth medium contains FBS, please wash once cells with FBS(-) medium.
2. Cells should be re-suspended at a concentration of  $1 \times 10^6$  cells/ mL in pulsed medium (RPMI 1640 with 0.5% human serum albumin).
3. Add 1mL of cells into each test tube.
4. Add peptide at appropriate concentration, and incubate at 37°C, 5% CO<sub>2</sub> (>2 hours).

### 2) Flow cytometry

1. Collect cells by centrifugation at 400 × g for 5 minutes, and aspirate the supernatant.
2. Wash twice with 1 mL of FCM buffer (2% FBS/0.05% NaN<sub>3</sub>/PBS).
3. Re-suspend the cells with 50 μL of FCM buffer.
4. Add 10 μL of Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001). Mix well and incubate for 5 minutes at room temperature.

5. Add 10 μL of TCR Tetramer. Mix well and incubate for 30-60 minutes at 2-8°C protected from light.
6. Add any additional antibodies (e.g. anti-HLA-A24 antibody). Mix well and incubate for 30 minutes at 2-8°C protected from light.
7. Add 1 mL of FCM buffer.
8. Centrifuge tubes at 400 x g for 5 minutes, and aspirate the supernatant.
9. Suspend the pellet in 500 μL of FCM buffer. Analyze it immediately or suspend it in 0.5% paraformaldehyde/PBS and store the sample in a dark room at 2-8°C. Be sure to analyze it within 24 hours.

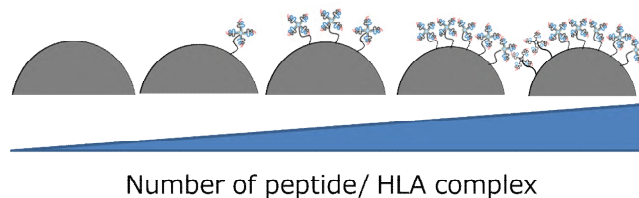
## Technical Hints

- A. Clear Back reagent (human FcR blocking reagent) may effectively block non-specific binding, resulting in clear staining when cells are stained with TCR Tetramer and antibodies. Please refer to the data sheet (MBL, code no. MTG-001) for details.
- B. Apoptotic, necrotic, and/or damaged cells are sources of interference in the analysis of viable cells by flow cytometry. Cell viability should be determined by 7-aminoactinomycin D (7-AAD) staining; intact viable cells remain unstained (negative).
- C. Cells do not require fixation prior to analysis if the stained cells are analyzed by flow cytometry within several hours.

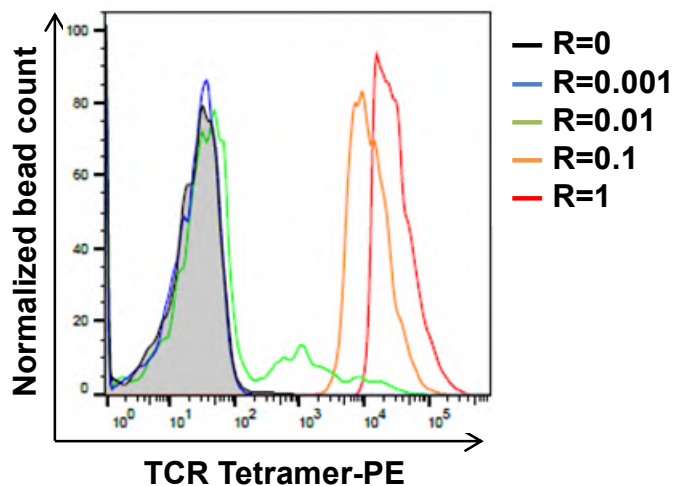
## Experimental Data

### 1) Bead-based Binding Assay

Streptavidin-coated beads were loaded with biotinylated peptide/HLA complexes at a dilution ratio ranging from  $10^{-3}$  to 1. These beads were stained with PE-conjugated TCR Tetramer and the PE-fluorescence intensity was measured by flow cytometry. Non-coated beads were used as a negative control.



The data reveal the staining of beads loaded with HLA-A\*24:02 survivin-2B<sub>80-88</sub> (AYACNTSTL) complex, using survivin-2B TCR Tetramer. A representative histogram indicates TCR Tetramer-positive populations in the normalized bead count. Reactivity of TCR Tetramers depends on the number of peptide/HLA complexes coated on the beads.



## 2) Cell Staining

HLA-A\*24:02 cells (HLA-A\*24:02 expressing C1R cells) were pulsed with or without survivin-2B<sub>80-88</sub> peptide (10 µg/mL, AYACNTSTL) in RPMI 1640 medium containing 0.5% human serum albumin for 4 hours. The cells ( $1.0 \times 10^6$  cells/test) were stained with survivin-2B TCR Tetramer and anti-HLA-A24 antibody, as described in the **Protocol**.

Numbers in the upper right quadrants represent the percentages of TCR Tetramer<sup>+</sup> cells relative to the total HLA-A24<sup>+</sup> cells.

