Dyemer and myDyemer T Cell Staining Reagents

Version 2.0_EN_RUO, 2024-06

RUO	For research use only!
Σ	50 tests
2°C 8°C	2...8°C
	See package printings

1. Introduction

1.1. Overview

This manual describes the protocol for the imusyn

Dyemer T Cell Staining Reagents

and

myDyemer T Cell Staining Reagents

for the detection of antigen-specific T cells by flow cytometry.

T cells play a central role in the immune system and are indicative of an individual's immune and health status. Studying T cell frequencies allows to identify determining events and factors that lead to alterations in individual T cell populations (e.g. by antigen exposure). Monitoring the changes in T cell frequencies is an effective tool in the field of immunology and infectiology. For the detection of antigen-specific T cells, soluble fluorophore-labeled peptide-MHC complexes (pMHC) are used. The recognition of these complexes by specific T cell receptors facilitates the quantification and enumeration of antigen-specific T cells in a variety of biological samples.

1.2. Test Principle

MHC class I complexes carrying a specific antigenic peptide are recognized by their cognate TCR. Dimerization of pMHC complexes onto fluorophore-labeled streptavidin allows T cell surface staining and facilitates the detection and quantification of antigen-specific T cells in flow cytometry application.

1.3. Statement of Intended Use

This reagent is for research use only.

2. Materials and Equipment

2.1. Definition of Symbols

Dyemer MHC class I loaded with a specific peptide and bound to PE-labeled streptavidin. 1 ml total volume, 50 tests

myDyemer Loadable "peptide-receptive" MHC class I bound to PE-labeled streptavidin. 1 ml total volume, 50 tests

Buffer SW Stain and Wash Buffer, phosphate buffered saline (PBS) + 2% FCS, pH 7.4

Buffer BE Biotin Elution Buffer, phosphate buffered saline (PBS) + 2% FCS + 1mM Biotin, pH 7.4

2.2. Storage and Expiry Date

Store at 2…8°C. Avoid exposure to light. If the storage conditions are met, the product can be used until the expiration date given on the primary packaging and the certificate of analysis.

2.3. Materials Supplied by the User

- Stain and Wash Buffer Buffer SW
- Biotin Elution Buffer Buffer BE
- Fluorophore-labeled antibodies for detection of relevant surface markers (e.g. CD3, CD8, …)
- Test tubes
- Optional: peptide solution (2 mg/ml) for preparation of myDyemers

3. Preparation and Usage

Contaminations have to be avoided during all steps.

3.1. Optional: Preparation of myDyemer

myDyemer allows flexible loading of specific peptides onto MHC class I molecules, pre-multimerized to PE-labeled streptavidin.

1. Peptide loading: Per test add 0.75 µl of peptide (2 mg/ml) to 19.25 µl of peptide-receptive myDyemer. Incubate over night at 2…8°C.
2. Loaded myDyemers can be used in flow cytometry application directly (see chapter 3.2) or stored long-term at 2…8°C for later use.

3.2. Standard PBMC Staining Protocol

1. Thaw or prepare PBMCs according to your protocol.
2. Optional: Wash cells with 10 ml Buffer SW by centrifugation at 250 x g for 10 min to remove any residues of biotin-containing culture medium.
3. Resuspend PBMCs and dilute to 1 x 10^7 cells/ml in Buffer SW.
4. Transfer 1 x 10^6 PBMCs (100 µl) to each test tube.[1]
5. Add 20 µl of Dyemer or loaded myDyemer to the cells and vortex briefly.
6. Incubate for 30 min at room temperature in the dark.
7. Add antibodies as recommended by the provider.[2]
8. Incubate for 30 min at 2…8°C in the dark.
9. Wash cells by adding 2 ml of Buffer SW. Centrifuge at 300 x g for 5 min at room temperature. Repeat wash step once.
10. Resuspend the cell pellet in a minimum of 200 µl Buffer SW.
11. Analyse directly or store at 2…8°C in the dark until proceeding to flow cytometry or cell sorting (do not store for more than 1 hour prior to analysis).

⚠ Do not include empty myDyemers as negative control. Instead, use myDyemers loaded with a peptide that is non-reactive to the $CD8^+$ T cell population in your sample.

3.3. Optional: Removal of Dyemer/myDyemer from cells

1. Perform cell sorting according to your protocol.
2. Pellet cells by centrifugation at 300 x g for 5 min at room temperature. Discard supernatant.
3. Elute Dyemer / myDyemer by adding 200 µl of Buffer BE. Incubate for 10 min at room temperature. Centrifuge at 300 x g for 5 min at room temperature. Repeat elution step once.
4. Resuspend cells in an appropriate amount of buffer or cell culture medium.

[1] Dyemer and myDyemer are stored in a buffer containing sucrose. Note that sucrose may be toxic to cells at certain concentrations. Be sure to dilute the cell suspension to 100 µl before adding the reagent.

[2] It is recommended to perform antibody addition after Dyemer incubation. Certain anti-CD8 antibody clones may interfere with MHC:TCR interaction.

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4. Troubleshooting and Performance

4.1. Troubleshooting

Problem	Possible cause	Solution
High Background / Non-Specific Staining	High rate of dead or aggregated cells	Use a cell viability dye (e.g. 7-AAD).
		Perform doublet discrimination.
	Unspecific binding to non-T cells	Use surface markers to exclude non-T cells (e.g. CD19 for gating out B Lymphocytes).
	Protein aggregates	Centrifuge Dyemers/myDyemers at 25.000 x g for at least 10 min at 4°C before staining.
	Prolonged incubation time	Incubation for longer periods than recommended may contribute to non-specific staining.
Low Signal / Insufficient Staining	Anti-CD8 antibody interference with MHC:TCR interaction	Check for anti-CD8 clones recommended for T cell staining (e.g. HIT8a or T8).
	Low frequency of antigen-specific T cells.	Perform T cell enrichment before proceeding to the staining protocol.
		Verify the presence of antigen-specific T cells by alternative assays (e.g. ELISpot).

4.2. Exemplary Data

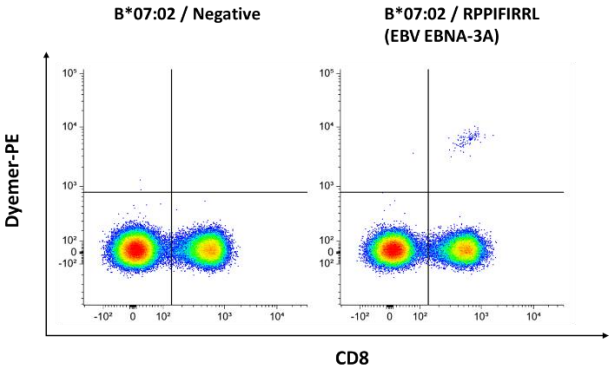

Figure 1: Representative data of T cell staining from healthy donor PBMCs. Human PBMCs ($1x10^6$) were gated for singlets, lymphocytes, $CD3^+$ and $CD8^+$ cells and stained with PE-labeled Dyemer HLA-B*07:02 (RPPIFIRRL) or Dyemer HLA-B*07:02 (Negative).

Changes to the previous version are highlighted.

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