CD19 CAR T-Cell detection reagent

PE Human protein CD19

REF	Σ		
19-0212-25T	25 test	PE	RUO
19-0212-100T	100 test		

1. INTENDED USE

Identification and quantification of CD19 CAR T-Cells by flow cytometry.

2. DESCRIPTION

Source: human CD19 protein (20-291), Fc Tag, expressed from human 293 cells (HEK) and subsequently fluorochrome-conjugated with a proprietary technology (BrightStep). MW: 56.6 kDa.
Purification: affinity chromatography.
Composition: human CD19 protein conjugated in an aqueous solution containing a stabilizing protein and 0.09% sodium azide (NaN3).
Recommended Usage: the recommended usage of this reagent is 5 µl for sample volumes ranging from 100 µl to 2 ml.

Protein Accession:	CD19 (Pro 20 - Lys 291)	Fc (Pro 100 - Lys 330)
	P15391-1	P01857

3. APPLICATION FIELD

Flow cytometry reagents play a crucial role in monitoring and evaluating the efficacy of CAR T-Cell therapies (chimeric antigen receptor T-cell therapies). These therapies represent a revolutionary approach in cancer treatment, where a patient's T cells are genetically modified to specifically recognize and attack cancer cells. However, to ensure the success of these therapies, closely monitoring the persistence and activity of CAR T-Cells in the patient's body is crucial.

The reagents used in flow cytometry are essential in this process. Their ability to provide precise identification and quantification of CAR T-Cells in blood or other tissues samples, as well as assessing their viability and function. This is critical to determine whether CAR T-Cells persist in the patient's body at sufficient levels to effectively combat cancer and whether they maintain their ability to recognize and destroy cancer cells.

Immunostep's CD19 CAR T-Cell is an antigen-based reagent consisting of the human CD19 extracellular domain (20-291) conjugated to fluorochrome using a proprietary technology (BrightStep) that enhances the detection of CAR T-Cells.

4. PRINCIPLE OF METHODOLOGY

Immunostep's CD19 CAR T reagent has been developed for the detection of transduced T-cells that are engineered to express CD19-specific chimeric antigen receptor (CAR) on the cell surface by direct immunofluorescence flow cytometry protocol.

5. STORAGE AND HANDLING CONDITIONS

Store refrigerated between +2 and +8°C. DO NOT FREEZE.

The unopened kit is stable until the expiry date indicated on the vial. Do not use after this date. After opening, reagents are stable if stored at +2 to +8°C and protected from contamination.

6. MATERIALS, REAGENTS AND EQUIPMENT REQUIRED NOT SUPPLIED.

- Flow cytometer equipped with at least one blue laser, 488 nm, and fluorescent channels for PE (Ex-Max 496 nm/Em-Max 578 nm).
- Centrifuge.
- Vortex Agitator
- Adjustable calibrated micropipettes covering a range of 1-1000 µL and corresponding disposable pipette tips.
- Pipette tips.
- 12x75 mm Polystyrene round-bottomed tubes (Cytometer tubes).
- Phosphate buffered saline (PBS) with 0.09% sodium azide. It is recommendable to add 0.5% BSA.
- Lysing solution. (Ref# RBC10X-50M), 10X concentrated solution containing ammonium chloride (NH_4Cl). For use dilute the concentrated RBC Lysis Solution (1:10) (5mL de RBC Lysis Solution 10X +45mL distilled water).
- Timer.
- Disposable gloves.
- Waste container for biological substances.
- Antibody panel for CAR T-Cell identification:

PRODUCT DESCRIPTION	REF
OC515 Anti-human CD45	45OC2-100T
FITC Anti-human CD3	3F1-100T
PerCP-Cy5.5 Anti-human CD4	4PP-100T
APC Anti-human CD8	8A1-100T
PE-Cyanine7 Anti-human CD45RA	45RAPC7-100T

Table 1: Antibodies which can be used for assessment of CAR T-Cells in the peripheral blood (optional recomendation).

7. RECOMMENDATIONS AND WARNINGS

⚠ Co-staining with anti-human CD19 antibodies should be avoided as the CD19 CAR T-Cell detection reagent can also recognized by CD19 antibodies.

⚠ Before acquiring samples, it is necessary to ensure that the flow cytometer settings and their compensation are appropriate.

⚠ The reagents contain sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds should be dissolved with tap water before disposal. These conditions are recommended to avoid deposits in pipes. Material Safety Data Sheet (MSDS) available on the website *www.immunostep.com.*

⚠ Keep kit components away from direct light exposure during the protocol. Fluorescently conjugated reagents are sensitive to light.

⚠ Wear personal protective equipment for sample handling. Wash hands properly after handling specimens. All procedures should be carried out in accordance with approved safety standards.

8. SAMPLE COLLECTION

The extraction of blood samples should be carried out in blood collection tubes using the appropriate anticoagulant (EDTA or heparin). For optimum results, the sample should be processed during the six hours following the extraction. Samples which cannot be processed within the 48 hours following the extraction should be discarded.

9. PROTOCOL

9.1 FACS Lysis Protocol

Protocol for immunofluorescent staining. The protocol can be carried out in a cytometer tube (12x75 mm) (ANNEX I):

1. Prepare a staining cocktail containing the volume indicated on the CD19 CAR T-Cell reagent (CD19p) vial and additional conjugated antibodies. See examples in Table 1. For more details, please refer to the respective data sheets.
2. Add 100 µL of sample (up to 10^6 cells) and mix properly in the vortex.
3. Incubate in the dark for 30 minutes at room temperature (20-25°C) or for 60 minutes at 4°C.
4. Add 2 ml of the lysing solution, mix in the vortex and incubate in the dark for 10 minutes or until the sample is lysed.
5. Centrifuge at 540g for 5 minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet. Leave 50 µl of unaspirated liquid to facilitate pellet resuspension.
6. Add 2 ml of PBS (please see materials required but not provided).
7. Centrifuge at 540g for 5 minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet. Leave 50 µl of non-aspirated liquid.
8. Resuspend the pellet in 0.3 ml of PBS.
9. Acquire on a flow cytometer or store in the dark at 2°C -8°C until the analysis is carried out. Samples should be acquired within 1 hour after lysis.

9.2 Suggested Bulk Lysis Protocol

1. Transfer no more than 2 ml of the sample to a 50 ml tube.
2. Fill the tube up to reach 50 ml volume using the diluted RBC Lysis Solution (Ref# RBC10X-50ML).
3. Shake and incubate for 15 minutes on a laboratory roller mixer. (Note: Observe turbidity to evaluate red blood cell lysis. Once the sample becomes clear, lysis is complete.
4. Centrifuge for 10 minutes at 800 xg speed. Decant supernatant.
5. Add 2 ml Phosphate buffered saline (PBS) and resuspend the cellular pellet and fill up to 50 ml with the same PBS.
6. Centrifuge for 10 minutes at 800 xg speed. Decant supernatant.
7. Add 800 µl PBS and resuspend the cellular pellet.
8. In four cytometers tubes (12x75 mm), prepare a staining cocktail containing the volume indicated on the CD19 CAR T-Cell reagent (CD19p) vial and additional conjugated antibodies. See examples in Table 1. For more details, please refer to the respective data sheets.
9. Transfer the cell suspension to four cytometers tubes (12x75 mm) containing the staining cocktail, 200 µ l in each tube.
10. Incubate in the dark for 30 minutes at room temperature (20-25°C) or for 60 minutes at 4°C.
11. Add 2 ml of PBS (please see materials required but not provided).
12. Centrifuge at 540g for 5 minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet. Leave 50 µl of non-aspirated liquid.
13. Resuspend the pellet in 0.3 ml of PBS.
14. Acquire on a flow cytometer or store in the dark at 2°C -8°C until the analysis is carried out. Samples should be acquired within 1 hour after lysis.

10. CYTOMETER ASSAY ACQUISITION AND ANALYSIS STRATEGY

It is essential in multicolor flow cytometry to correct fluorescence spillover. Each laboratory must establish their own procedures for verifying that compensation is appropriate for your antibody panel.

Use the same reagent as was used for the fully stained sample so the spectra match identically.

10.1 Strategy for analysis of samples

A first step of screening the nucleated cell population in the FSC-H/FSC-A pattern to remove to remove residual debris and reduce background is recommended, followed by gating the nucleated cell population in the SSC-A/FSC-A dot plot to remove doublets, allowing correct identification of the lymphocyte population (A). On a dot plot for the following CD45/CD3 gating (B), for selection of the T-lymphocyte population. Finally, in a dot plot for the CAR T-Cell marker (CD19 CAR T-Cell reagent), for the gating of CAR T-Cells. In this dot plot CD45RA is only used to further define the gating of the CAR T-Cell population.

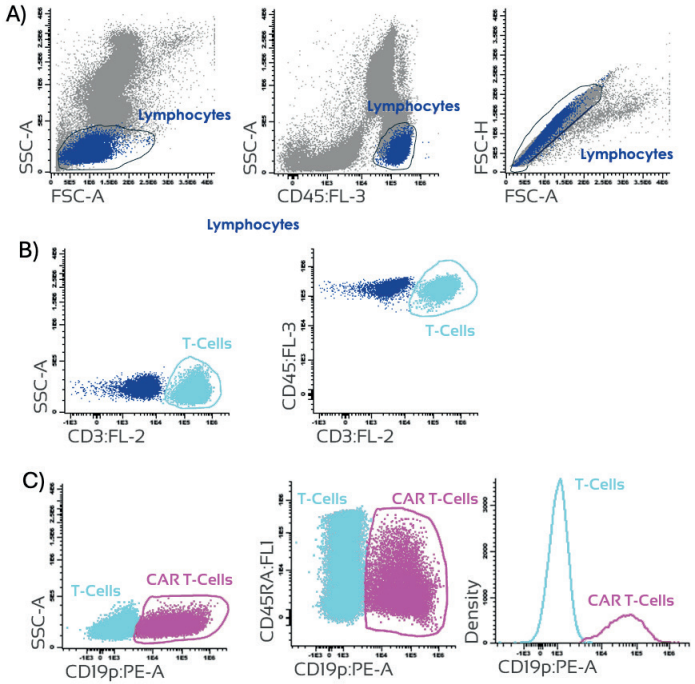

Figure 1: Analysis strategy for CAR T-Cell population selection in the FSC-H/FSC-A (A); SSC or CD45/CD3 (B) and pCD19 (C) dot plots.

11. WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties.

Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

12. ADDITIONAL INFORMATION

For research use only. Not for diagnostic use.

Not for resale. Immunostep will not be responsible of violations that may occur with the use of this product. Any use of this product other than the specified in this document is strictly prohibited.

Unless otherwise indicated by Immunostep by written authorization, this product is intended for research only and is not to be used for any other purpose, including without limitation, for human or animal diagnostic, therapeutic or commercial purposes.

Please, refer to www.immunostep.com technical support for more information.

13. EXPLANATION OF SYMBOLS

	Fluorochrome
REF	Product reference
Σ	Content for <n> analysis
	Regulatory Status
RUO	Research Use Only
	Expiry date
LOT	Batch code
	Follow the instructions for use
	Preservation from x°C to y°C
	Pay attention to
	Contents of each test
	Manufacturer

14. MANUFACTURER'S DATA

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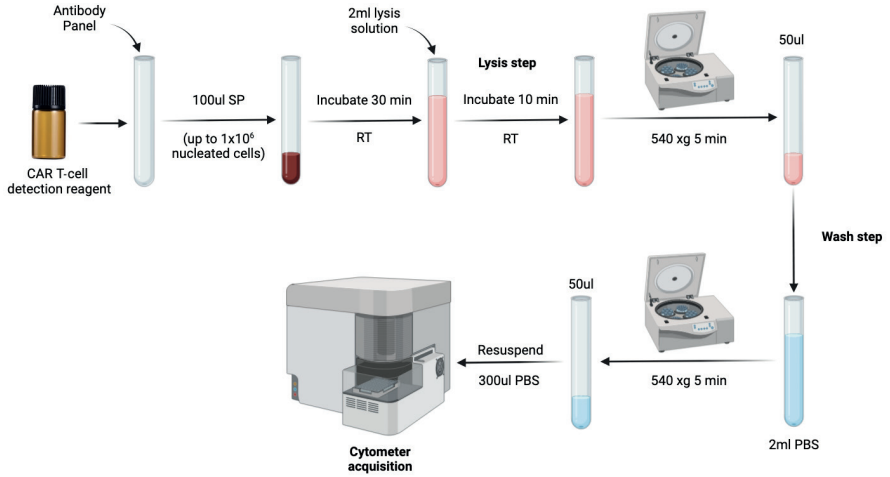
ANNEX I. Protocol for immunofluorescent staining



ANNEX II. Suggested Bulk Lysis Protocol