

# Anti- Human T CR C $\beta$ 2 (SAM.2.rMAb)

	REF	$\Sigma$		[A]	
PE	TCRCB2PE	100 test	2 $\mu$ l / test	0,5 mg/ml	
APC	TCRCB2A	100 test	2 $\mu$ l / test	0,5 mg/ml	
PE	TCRCB2PE	100 test	2 $\mu$ l / test	0,25 mg/ml	

## 1. PRODUCT DESCRIPTION

**Clone:** SAM.2.rMAb

**Isotype:** Mouse IgG1,k

**Tested application:** flow cytometry

**Species reactivity:** Human (QC Testing)

**Storage instruction:** store in the dark at 2-8 °C

**Storage buffer:** aqueous buffered solution containing protein stabilizer and 0.09% sodium azide ( $\text{NaN}_3$ ).

**Recommended usage:** Immunostep's anti-human T cell receptor (TCR) antibody, clone SAM.2.rMAb, is a monoclonal antibody designed for the identification and enumeration of TCR C $\beta$ 2, a member of the immunoglobulin superfamily. This antibody targets a constant region determinant found on the surface of all TCR C $\beta$ 2-bearing T lymphocytes. The TCR C $\beta$ 2, in conjunction with CD3, forms the CD3/TCR complex, which is crucial for T cell and thymocyte function. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 1 test for  $10^6$  cells.

**Presentation:** liquid

**Source:** Supernatant proceeding from an in vitro cell culture of a cell hybridoma.

**Purification:** Affinity chromatography.

## 2. ANTIGEN DETAILS

**Large description:** The SAM.2.rMAb is a recombinant monoclonal antibody that specifically recognizes the TCR C $\beta$ 2 constant region, which is expressed by a significant proportion of CD4+ and CD8+ T cells. Thymocytes and mature peripheral T cells predominantly express a heterodimeric T cell receptor (TCR  $\alpha\beta$ ) for antigen recognition, which is comprised of disulfide-linked transmembrane  $\alpha$  and  $\beta$  chain subunits. The constant region of the TCR  $\alpha$  subunit is encoded by the TRAC gene, whereas the TCR  $\beta$  subunit is encoded by either of two highly homologous constant region genes: TCRB1 for TCR C $\beta$ 1 or TCRB2 for TCR C $\beta$ 2.

The JOVI.1 antibody (ref. JOVIF) alternatively recognizes the TCR C $\beta$ 1 constant region expressed by the other subset of TCR  $\alpha\beta$ + T cells. These antibodies, SAM.2.rMAb and JOVI.1, are effectively used together in multicolor staining and flow cytometric analyses to identify and characterize the nature of TCR C $\beta$ 1+ or TCR C $\beta$ 2+ T cells within heterogeneous cell population.

The TCR  $\alpha\beta$  complex plays a crucial role in the adaptive immune response by recognizing peptide antigens presented by major histocompatibility complex (MHC) molecules on antigen-presenting cells. This recognition leads to T-cell activation and subsequent immune responses. The constant regions of the TCR chains are essential for maintaining the structural integrity and proper function of the receptor.

It is important to note that some human CD3- and TCR-specific antibodies might not be compatible for co-staining human T cells with SAM.2.rMAb. Therefore, careful selection and validation of antibodies are necessary to ensure accurate and reliable results in flow cytometric analyses.

## 3. WARRANTY

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## 4. REFERENCES

1. Berg H, Otteson GE, Corley H, et al. Flow cytometric evaluation of TRBC1 expression in tissue specimens and body fluids is a novel and specific method for assessment of T-cell clonality and diagnosis of T-cell neoplasms. *Cytometry B Clin Cytom.* 2021; 100(3):361-369.
2. Ferrari M, Baldan V, Ghongane P, et al. Targeting TRBC1 and 2 for the treatment of T cell lymphomas. *Abstract. Cancer Res.* 2020; 80:2183.
3. Horna P, Shi M, Olteanu H, Johansson U. Emerging Role of T-cell Receptor Constant  $\beta$  Chain-1 (TRBC1) Expression in the Flow Cytometric Diagnosis of T-cell Malignancies. *Int J Mol Sci.* 2021; 22(4):1817.

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## 6. PROTOCOL

### Direct Immunofluorescence Cell Surface Staining Protocol

1. Transfer 100  $\mu$ l ( $10^6$  cells/test) of the sample to a 12 x 75 mm polystyrene test tube.
2. Add the suggested volume indicated on the antibody vial to the 12x75 mm cytometer tube.
3. Mix well and incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix. Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution).
5. Centrifuge tubes at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
6. Resuspend and wash with 3-5 mL of PBS at 540xg for 5 min.
7. After removing the supernatant and resuspending the cell pellet, add 300  $\mu$ L of PBS and acquire on the flow cytometer are recorded.
8. Analyse on a flow cytometer or store at 2- 8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis.

### Indirect Immunofluorescence Cell Surface Staining Protocol

1. Transfer 100  $\mu$ l ( $10^6$  cells/test) of the sample to a 12 x 75 mm polystyrene test tube.
2. Add purified reagent according to manufacturer's recommendation and mix gently with a vortex mixer.
3. Incubate in the dark at room temperature at 4 °C for 30 minutes or at room temperature (20-25 °C) for 15 minutes.
4. Add 2 mL 0.01 mol/L PBS (it betters that it containing 2% bovine serum albumin) and resuspend the cells by using a vortex mixer. Centrifuge at 540xg for 5 min in order to remove the McAb not bound to its antigen.
5. Add a secondary conjugated reagent with some fluorochrome and mix. Incubate at room temperature for 15 min in the dark. The absence of light is necessary as the fluorochrome is photoinstability.
6. After the incubation period, add 1,5 ml of an erythrocyte-lysing solution and mix. Incubate at room temperature in the darkness (the blood should be well mixed with the lysing solution). Centrifuge at 540xg for 5 minutes. The supernatant is removed with a Pasteur pipette or with a vacuum pump.
7. Resuspend and a made a final wash with 3-5 mL of PBS at 540xg for 5 min.
8. After removing the supernatant and resuspending the cell pellet, add 300  $\mu$ L of PBS and acquire on the flow cytometer are recorded.
9. Analyse on a flow cytometer or store at 2- 8 °C in the dark until analysis. Samples can be run up to 24 hours after lysis

## 7. EXPLANATION OF SYMBOLS



Form



Catalog reference



Contains sufficient for <n> test



Regulatory Status



Quantity per test



Research Use Only



Concentration



Manufacturer

## 8. MANUFACTURED BY:



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