

Anti-Human TCR C β 1 (JOVI.1)

Fluorochrome	Reference	Test
FITC	JOVIF	100 test



PRODUCT DESCRIPTION

Other Names: Anti-Beta T Cell Receptor; TCRB; TRBC1; anti-TCR Cbeta1; a; TCRcb1; TRBC1; TRBC-1.

Description: anti-Human TCR C β 1 monoclonal antibody is derived from the hybridization of myeloma cells and mouse spleen cells immunized with T cells of a transgenic mouse line expressing human TcR V2 β C β 11. The antibody is made up of an IgG2a heavy chain and a kappa light chain.

Clone: JOVI-1

Isotype: Mouse IgG2a, kappa

Reactivity: Human

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

Purification: Affinity chromatography.

Composition: anti-human TCR C β 1 monoclonal antibody conjugated with FITC and in an aqueous solution which contains stabilising protein and 0.09% sodium azide (NaN₃).

Fluorochrome	Reagent provided	Concentration (mg/ml)
FITC (fluorescein isothiocyanate)	100 ug en 0,2 ml	0,5

RECOMMENDED USAGE

Immunostep's Anti-Human TCR C β 1 antibody is a conjugated monoclonal antibody for use *in vitro* diagnostics by flow cytometry for the identification of T-cell populations that express the C β 1 subunit of the TCR T-cell receptor, which represents the 50-75% of peripheral blood CD3 + lymphocytes in healthy individuals according to their general normal immunophenotype, being able to provide a clonality test in a similar way to the light chain restriction analysis for B3 lymphocytes. Anti-Human TCR C β 1 conjugated to a fluorochrome is a single color direct immunofluorescence reagent intended for the identification of T cells expressing the constant subunit beta1 (C β 1) of the T cell receptor (TCR).²

CLINICAL RELEVANCE

This antibody can be used alone or in combination with others for the diagnosis or prognosis of some hematological diseases such as T-cell large granular lymphocytic leukemia (T-LGLL), identification of Clonal T-cell large granular lymphocytic populations of uncertain significance (T-CUS), cutaneous T-cell lymphoma (CTCL) ^{2-4,8}.

PRINCIPLES OF THE TEST

The monoclonal anti-TCR C β 1 antibody binds to the surface of T cells that express the human T-cell β 1 receptor chain antigen.

To identify these cells, the sample is incubated with the antibody and analyzed on a flow cytometer.

APPROPRIATE STORAGE AND HANDLING CONDITIONS

Store in the dark, refrigerated between 2 °C and 8 °C. DO NOT FREEZE. The antibody is stable until the expiry date stated on the vial label if kept at 2°C-8°C. Do not use after the date indicated.

Once the vial is open, the product is stable for 90 days.

EVIDENCE OF DETERIORATION

Reagents should not be used if any evidence of deterioration is observed. For more information, please contact our technical service: tech@immunostep.com

The product's normal appearance is a semi-transparent, colourless liquid. It should not be used if liquid medium is cloudy or contains precipitate. It should be odourless.

RECOMMENDATIONS AND WARNINGS



- The reagents contain sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop. The safety data sheet (SDS) is available online at www.immunostep.com
- Avoid microbial contamination of the reagent.
- Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
- Never mouth pipette.
- In the case of contact with skin, wash in plenty of water.
- The samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed.
- Do not use after the expiry date indicated on the vial.
- Deviations from the recommended procedure could invalidate the analysis results.
- FOR *IN VITRO* DIAGNOSTIC USE.
- For professional use only.
- Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.
- In case of background, centrifuge the product at 2000 rpm for 2 minutes to avoid interferences.

SAMPLE COLLECTION

The extraction of venous blood samples should be carried out in blood collection tubes using the appropriate anticoagulant (EDTA or heparin)^{9,10,11}. 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.

MATERIALS REQUIRED BUT NOT PROVIDED

- Isotype controls:

Fluorochrome	Isotype control	Immunostep Reference
FITC	Mouse IgG2a	ICIGG2AF-100UG

- Centrifuge
- Commonly used 12 x 75-mm flow cytometry assay tubes
- Micropipettes for dispensing volumes from 5 µl to 2 ml
- Blood collection tubes with anticoagulant.
- Phosphate buffered saline (PBS) with 0.09% sodium azide. It is recommendable to add 0.5% BSA
- Vacuum system
- Lysing solution
- Flow cytometer equipped with laser and appropriate fluorochrome filters
- Vortex Agitator

SAMPLE PREPARATION:

1. Add the suggested volume indicated on the antibody vial to a 12x75-mm cytometer tube. It is advisable to prepare an additional tube with the appropriate isotype control (*please see materials required but not provided*).
2. Add 100 µL of sample (up to 10⁶ cells) and mix properly in the vortex.
3. Incubate in the dark for 15 minutes at room temperature (20-25°C) or for 30 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 five minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet. Leave 50 µl of non-aspirated liquid.
6. Resuspend pellet.
7. Add 2 ml of PBS (*please see materials required but not provided*).
8. Centrifuge at 540g for five minutes and carefully withdraw the supernatant by suction so as not to touch the cell pellet. Leave 50 µl of non-aspirated liquid.
9. Resuspend the pellet in 0.3 ml of PBS.

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 the 3 hour after lysis.

FLOW CYTOMETRY ANALYSIS

Collect the fluorescence attributed to monoclonal antibody and determine the percentage of stained cells. It is necessary to use an isotope control conjugated with the same fluorochrome, of the same type of immunoglobulin heavy chain and concentration to evaluate and correct the unspecific binding of lymphocytes (*please see materials required but not provided*). Set an analysis region to eliminate fluorescence background noise and to include positively stained cells.

Below is an example diagram of peripheral blood stained:

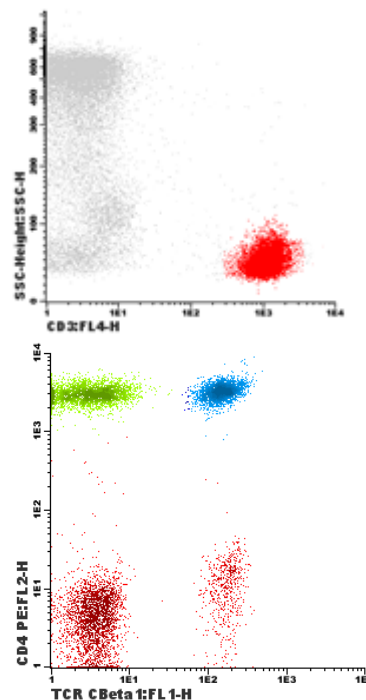


Fig. 1: Above a biparametric representation of the mean fluorescence intensity of the CD3 + lymphocyte population and its internal complexity (SSC) in a peripheral blood sample from a healthy donor. Below is the representation of the same sample with the selection of CD3 + lymphocytes confronting the expression of CD4 with the Cb1 TCRs.

LIMITATIONS OF THE PROCEDURE

1. Incubation of antibody with cells for other than the recommended procedures may result in a reduction or loss of antigenic determinants from the cell surface.
2. The values obtained from normal individuals may vary from laboratory to laboratory; it is therefore suggested that each laboratory should establish its own normal reference range.
3. Abnormal cells or cell lines may show a higher antigen density than normal cells. In some cases, this could require the use of a greater quantity of monoclonal antibody than is indicated in the procedures for sample preparation.
4. In whole blood samples, red blood cells found in abnormal samples, as well as nucleated red cells (from both normal and abnormal specimens) may be resistant to lysis. Longer periods of red blood cell lysing may be needed in order to avoid the inclusion of unlysed cells in the lymphocyte gated region.
5. Blood samples should not be refrigerated for an extensive period (more than 24 hours), since the number of viable cells will gradually decrease, and this may have an effect on the analysis. In order to obtain the best values, they should be kept at room temperature immediately prior to incubation with the monoclonal antibody.

6. Accurate results with flow cytometric procedures depend on correct alignment and calibration of the lasers, as well as correct gate settings.

REFERENCE VALUES

Abnormal results in the percentage of cells expressing the antigen or in its levels of expression may be due to pathological conditions. It is advisable to know the normal antigen expression patterns in order to ensure a proper interpretation of the results.⁽⁵⁻⁷⁾

The values obtained from healthy individuals may vary from laboratory to laboratory; it is therefore suggested that each laboratory should establish its own normal reference range.

CHARACTERISTICS

SPECIFICITY

The anti-Human antibody TCR C β 1 clone JOVI.1 is specific against different TcR β regions of all T-cell lines that express TcR C β 1, and does not recognize the HPB-ALL T-cell line that expresses TCR V β 5C β 2, however, a transfected T cell line and a TcR clone that express C β 2 TcR show reactivity with the antibody although this is very weak.

The JOVI-1 monoclonal antibody reacts with 50-75% of CD3 + lymphocytes from peripheral blood, although the marking profile is significantly different in the CD4 and CD8 subpopulations. Regarding the CD8 lymphocytes of peripheral blood of healthy individuals, the JOVI-1 antibody recognizes two different subpopulations in terms of their expression, the high JOVI-1 and the low JOVI-1, while for the population of peripheral blood lymphocytes CD4 +, lymphocyte marking is more heterogeneous. The variation in the intensity level of the JOVI-1 labeling is not a consequence of the down regulation of TcR levels in a proportion of CD4 + cells because this population expresses uniformly high levels of CD3.

Therefore, it is not clear which epitope in the TcR is recognized by JOVI-1. It is possible that a supertypic determinant is recognized in the V β domains or a V β epitope that is influenced by the particular V α associated with the V β domain. Another explanation is that JOVI.1 can recognize a C β determinant that is masked in some T cells by TcR interactions with other proteins. In this context, it is clear that TcR forms dynamic associations on the cell surface with a number of other surface structures.

However, this explanation is not consistent with the observation that JOVI-1 was unable to immunoprecipitate TcR from HPB-ALL cell detergent extracts when weak protein-protein associations are expected to be broken.

Further work will be necessary to establish the molecular basis of the JOVI-1.¹ antibody interactions.

LINEARITY

For the linearity analysis, different dilutions were made of a sample consisting of Jurkat T cell line that expresses a V β 8C β 1 TCR and Pre-B Nalm-6 cell line that does not express any TCR in the same way. that the total cell number e (1x10⁶ cells) and the sample volume remained constant. For each dilution, 3 replicates were made.

The data obtained is shown in the following table:

R	R square	Lineal regression
0,997	0.994	Y=1.030X + 0.928

ACCURACY

Accuracy was made according to the recommendations of the CLSI (The Clinical & Laboratory Standards Institute), the following scheme was designed: 3 sites X 5 days X 5 runs X 3 (batches) X 1 (sample formed by a pool of 3 samples).

Data acquisition was carried out on a FACS Calibur cytometer (BD Bioscience) and the percentage of CD4 TCR C β 1 positive lymphocytes was analyzed.

The reagent accuracy results are shown in the following table:

	*Repeatability(Whitin-run)	**Interseries (Between-run)	***Interday (between-day)	Reproducibility ****
N	25,0000	5,0000	5,0000	225,0000
Medium value	40,2912	39,5140	39,2860	40,2376
SD	1,1409	0,7484	0,9434	1,2073
CV (%)	2,8317	1,8939	2,4014	3,0003

* Repeatability (Whitin-run) was calculated with data from one laboratory and one batch.

** The interseries repeatability (Between-run) was calculated with the data obtained from a laboratory, a batch and a day.

*** The inter-day repeatability (Between-day) was calculated with the data obtained from a laboratory, a batch and a race.

**** The reproducibility includes all the data obtained from the 3 laboratories, the 5 days, the 5 runs and the 3 batches.

REPRODUCIBILITY

In the same assay, we analyzed batch-to-batch reproducibility and inter-laboratory and intra-laboratory precision. The laboratories in which the test was carried out were physically separated and had independent technicians and instruments.

To calculate reproducibility, the percentage of positive cells was analyzed.

The result of the analysis is shown in the following table:

	*(Between n-batch)	** (Between n-lab)	***Intra-laboratory Lab1	***Intra-laboratory Lab2	***Intra-laboratory Lab3
N	15,0000	75,0000	25,0000	25,0000	25,0000
Medium value	40,4307	40,2980	39,9620	40,2912	40,0364
SD	1,1920	1,1909	0,8289	1,1409	1,2765
CV (%)	2,9482	2,9551	2,0743	2,8317	3,1883

* The reproducibility between batches (Between-batch) was obtained with the data obtained by a laboratory during one day.

** Inter-laboratory reproducibility (Between-lab) was obtained with data from a batch, therefore it encompasses the precision between runs and the precision between days.

*** The intra-laboratory reproducibility was obtained with all the data obtained in the study by each laboratory, therefore it includes the precision between runs and the precision between days per laboratory.

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.

REFERENCES

1. Viney JL, Prosser HM, Hewitt CR, Lamb JR, Owen MJ. Generation of monoclonal antibodies against a human T cell receptor beta chain expressed in transgenic mice. *Hybridoma*. 1992 Dec;11(6):701-13. doi: 10.1089/hyb.1992.11.701. PMID: 1284120.
2. Shi M, Olteanu H, Jevremovic D, He R, Viswanatha D, Corley H, Horna P. T-cell clones of uncertain significance are highly prevalent and show close resemblance to T-cell large granular lymphocytic leukemia. Implications for laboratory diagnostics. *Mod Pathol*. 2020 Oct;33(10):2046-2057. doi: 10.1038/s41379-020-0568-2. Epub 2020 May 13. PMID: 32404954.
3. Horna P, Shi M, Olteanu H, Johansson U. Emerging Role of T-cell Receptor Constant β Chain-1 (TRBC1) Expression in the Flow Cytometric Diagnosis of T-cell Malignancies. *Int J Mol Sci*. 2021 Feb 12;22(4):1817. doi: 10.3390/ijms22041817. PMID: 33673033; PMCID: PMC7918842.
4. Shi M, Jevremovic D, Otteson GE, Timm MM, Olteanu H, Horna P. Single Antibody Detection of T-Cell Receptor $\alpha\beta$ Clonality by Flow Cytometry Rapidly Identifies Mature T-Cell Neoplasms and Monotypic Small CD8-Positive Subsets of Uncertain Significance. *Cytometry B Clin Cytom*. 2020 Jan;98(1):99-107. doi: 10.1002/cyto.b.21782. Epub 2019 Apr 11. PMID: 30972977.
5. Clinical applications of flow cytometry: Quality assurance and immunophenotyping of lymphocytes; approved guideline (1998). Wayne PA: National Committee for Clinical Laboratory Standards; Document H42-A.
6. Kotylo PK et al. Reference ranges for lymphocyte subsets in pediatric patients. *Am J Clin Pathol* 100:111-5 (1993)
7. Reichert et al. Lymphocyte subset reference ranges in adult Caucasians. *Clin Immunol Immunopathol* 60:190-208 (1991).
8. Noemí Muñoz-García et al. Anti-TRBC1 Antibody-Based Flow Cytometric Detection of T-Cell Clonality: Standardization of Sample Preparation and Diagnostic Implementation. *Cancers*. 2021, 13(17), 4379.
9. Procedures for the collection of diagnostic blood specimens by venipuncture- approved standard; Fifth edition (2003). Wayne PA: National Committee for Clinical Laboratory Standards; Document H3-A5.

10. Standard Procedures for the Collection of Diagnostic Blood Specimens", publicado por el National Committee for Clinical Laboratory Standards (NCCLS)
11. Clinical applications of flow cytometry: Quality assurance and immunophenotyping of lymphocytes; approved guideline (1998). Wayne PA: National Committee for Clinical Laboratory Standards; Document H42-A.

MANUFACTURED BY



Immunostep S.L

Avda. Universidad de Coimbra, s/n
Cancer Research Center (CIC)
Campus Miguel de Unamuno
37007 Salamanca (Spain)
Tel. (+34) 923 294 827
www.immunostep.com