Anti-Human CD3/CD8/CD45 (33-2A3/143-44/D3/9)

Fluorochrome	Reference	Test
FITC/PE/PerCP	3FI8PE145PP1-50T	50 tests

IVD

PRODUCT DESCRIPTION

Clone: 33-2A3, 143-44, D3/9
Isotype: IgG2a, IgG1, IgG1
Tested application: flow cytometry
Immunogen: The anti-CD3 monoclonal antibody derives from human leukocytes. The anti-CD8 monoclonal antibody derives from T cells. The anti-CD45 monoclonal antibody derives from T cells from leukemic HPB-ALL.
Species reactivity: Human
Storage instruction: store in the dark at 2-8 °C **Storage buffer:** aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN3). Recommended usage: Immunostep's CD3/CD8/CD45, is a monoclonal antibody intended for simultaneous detection and enumeration of lymphocytes. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 1 test for 106 cells.
Presentation: liquid
Source: Supernatant proceeding from an in vitro cell culture of a cell hybridoma.
Purification: Affinity chromatography.

ANTIGEN DETAILS

Large description: The CD3 monoclonal antibody is directed against the CD3- antigen (T3-antigen), which is expressed on human T lymphocytes. The monoclonal antibody reacts with 80-90% human peripheral T lymphocytes and medullary thymocytes. The monoclonal antibody does not react with B-cells, monocytes, granulocytes and platelets. The monoclonal antibody is mitogenic for resting T lymphocytes and it blocks the cytolytic activity of CTL clones.

The CD8 monoclonal antibody is directed against the CD8-antigen (T8-antigen),which is expressed on human T lymphocytes. The monoclonal antibody reacts with 20-30% of human peripheral T lymphocytes. The monoclonal antibody reacts with T lymphocytes with suppressor-cell activity in pokeweed mitogen-stimulated immunoglobulin production, as was shown in separation experiments (i.e."panning").

The CD45 monoclonal antibody is directed against the CD45- antigen, defined T200 or Leucocyte Common Antigen. The antibody reacts with all cells of the haemopoietic lineage, not with cells of other lineages.

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

INSTRUCTIONS FOR USE

Store Diluting RBCX10 Lysis Solution:
1. Dilute the 10X RBCX10 concentrate 1:10 with room temperature (20–25 °C) deionized water.
2. The prepared solution is stable for 1 month when stored in a glass or high-density polyethylene (HDPE) container at room temperature.

Performing Quality Control: In accordance with the College of American Pathologists (CAP) guidelines, we recommend running two levels of liquid control material (process control).
Controls should be run at least once each day that patient testing is performed. Use commercial controls providing established values for percent positive and absolute counts with each run to assess system performance. Immunostep recommends using CDChex
Plus (Streck) normal and CD4 Low as process controls.

To perform quality control: Thoroughly mix the appropriate CD-Chex Plus (Streck) control, or equivalent process control. See the IFU for the control for detailed instructions.

1. Stain the control sample using Immunostep's CD3/CD8/CD45/CD4 antibody as described in te following section. The control sample should be processed like patient samples to monitor the ongoing performance of the entire analytic process.
2. Acquire the stained control sample on the flow cytometer.
3. Visually inspect the CD45 vs SSC dot plot. The lymphocyte population should appear as a bright, compact cluster with low SSC. Monocytes and granulocytes should also appear as distinct clusters. Do not proceed with analysis if populations are diffuse and there is little or no separation between clusters.
4. Verify that the results are within the values reported on the Assay Values sheet.

Staining the Cells:
Use care to protect the tubes from direct light. Perform the procedure at room temperature. See Precautions and Interfering Conditions.
1. For each patient sample, label a 12 × 75-mm tube with the sample identification number. For absolute counts, label an STEPCOUNT counting tube in place of the 12 × 75-mm tube. Note: Before use, verify that the STEPCOUNT bead pellet is intact and within the metal retainer at the bottom of the tube. If this is not the case, discard the STEPCOUNT counting tube and replace it with another.
2. Pipette 20 µL of Immunostep's CD3/CD8/CD45/CD4 antibody into the bottom of the tube. Note: If using an STEPCOUNT counting tube, pipette the reagent onto the side of the tube, just above the metal retainer, without touching the bead pellet.
3. Pipette 50 µL of well-mixed, anticoagulated whole blood into the bottom of the tube. Note: If using an STEPCOUNT counting tube, we recommend using the reverse pipetting technique to pipette the sample onto the side of the tube just above the metal retainer. Avoid smearing blood down the side of the tube. If whole blood remains on the side of the tube, it will not be stained with the reagent and can affect results.
4. Cap the tube and vortex gently to mix.
5. Incubate for 15–30 minutes in the dark at room temperature (20–25 °C).
6. Add 450 µL of 1X RBCX10 lysis solution to the tube.
7. Cap the tube and vortex gently to mix.
8. Incubate for 15–30 minutes in the dark at room temperature (20–25 °C). The sample is now ready to

be analyzed on the flow cytometer. If samples will not be analyzed immediately after staining, store them in the dark at room temperature (20–25°C).

Acquiring the Samples:

1. Vortex the cells thoroughly at low speed. It is important to reduce aggregation before running samples on the flow cytometer.Note: If you are using a Loader, vortex tubes immediately before placing them into the Loader racks.
2. Install the tube on the cytometer and acquire the sample. Before acquiring samples, adjust the threshold to minimize debris and ensure that populations of interest are included.
3. Analyze the data using the appropriate cytometer-specific software. See the cytometer's IFU for more information.

Results: Results are reported as the percentage of positive cells per lymphocyte population or as the number of positive cells per microliter of blood (absolute count).

Calculating Absolute Counts: During analysis, the absolute number (cells/µL) of positive cells in the sample can be determined by comparing cellular events to bead events. If Immunostep software is used, absolute counts will be determined by the software. For manual data analysis, the absolute

count of the cell population (A) can be calculated using the following equation:

$$A=X/Y\times N/V$$

Where:

- X is the number of positive cell events
- Y is the number of bead events
- N is the number of beads per test, which is found on the STEPCOUNT counting tubes foil pouch and can vary from lot to lot
- V is the sample volume (50 µL)

Controls:

- Positive Controls: Use CD-Chex Plus (Streck) normal and CD4 Low to validate the staining
- protocol. Negative Controls: Include isotype controls and unstained cells to set proper gating strategies.

Material Not Supplied:

- STEPCOUNT counting tubes (ref. 1399991218)
- RBCX10 lysis solution (ref. RBCX10-50ML)
- CD-Chex Plus (Streck) normal and CD4 Low

REFERENCES

1. McMichael AJ, Gotch FM. T-cell antigens: new and previously defined clusters. In: McMichael AJ, Beverley PCL, Cobbold S, Crumpton MJ, Gilks W, Gotch FM, et al., editors. Leucocyte typing III. White cell differentiation antigens. Proceedings of the 3rd International Workshop and Conference; 1986 Sept 21-26; Oxford, England. Oxford, New York, Tokyo: Oxford University Press; 1987. p. 31-62.
1. Tunnacliffe A, Olsson C, Traunecker A, Krissansen GW, Karjalainen K, de la Hera A. T3.2. The majority of CD3 epitopes are conferred by the epsilon chain. In: Knapp W, Dörken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, et al., editors. Leucocyte typing IV. White cell differentiation antigens. Proceedings of the 4th International Workshop and Conference; 1989 Feb 21-25; Vienna, Austria. Oxford, New York, Tokyo: Oxford University Press; 1989. p. 295-6

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