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Product: Three color reagents : CD4/CD8/CD3
Cat. Ref: 4F18PE13PC2-100T
Reagent provided: 100 test (15µl / test)
Description: Monoclonal Mouse Anti-Human CD4/CD8/CD3, is recommended for use in flow cytometry for simultaneous detection and enumeration Lymphocytes T CD4 and CD8. The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ⩽0.09% sodium Azide
Clones: HP2/6; 143-44; UCHT-1
Isotypes: IgG2a, IgG1, IgG1
Flororchromes: Fluorescein isothiocyanate (*FITC*), R-Phycoerythrin (*R-PE*) and PECy5.

Specificity: The CD4 monoclonal antibody is directed against the CD4-antigen (T4-antigen),which is expressed on human peripheral T lymphocytes and 80% of thymocytes. The monoclonal antibody reacts on a low level with human monocytes and macrophages. The monoclonal antibody does not react with B-cells ,granulocytes and thrombocytes.

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 monoclonal antiody does not react with B-cells, monocytes, granulocytes and platelets.

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.
HLDA: CD4 - 4th International Workshops on Human Leucocyte Differentiation, WS code 116
CD8 - 4th International Workshops on Human Leucocyte Differentiation, WS Code 169
CD3 - 3rd International Workshops on Human Leucocyte Differentiation, WS Code 208

Reactivity: The CD4 antibody recognizes 60Kd MW lymphocyte surface antigen identified by monoclonal antibodies belonging to the CD4 cluster and present on 54% of peripheral blood T lymphocytes, 50% of thymocytes and some malignant cells of T cell origin. Normal B lymphocytes, monocytes or granulocytes do not express surface CD4 antigen although cytoplasmic expression has been observed in monocytes/macrophages. The CD4 positive T lymphocyte subpopulation has been characterised functionally as comprising helper cells active in amplification of immune responses.

The CD8 antibody recognizes 30/32 kD MW lymphocyte surface antigen identified by monoclonal antibodies belonging to the CD8 cluster on a sub-population of peripheral blood T lymphocytes, 60% of thymocytes, and a limited number of malignancies of T cell origin. Normal B lymphocytes, monocytes or granulocytes do not express surface CD8 antigen.

Finally, CD3 antibody recognizes 22/26/30 kD MW lymphocyte surface molecules associated with the T cell antigen receptor complex. Reacts with 85% of peripheral blood T lymphocytes, 70% of thymocytes, the majority of T cell chronic lymphocytic leukaemias, Sezary leukaemias and approximately 70% of acute lymphoblastic leukaemias of T cell origin.

Clinical applications: Helper/inducer lymphocytes are a subset of T lymphocytes (CD3 +) that are CD4 + . CD3 + CD4 + counts are used to characterize and monitor some forms of immunodeficiency and autoimmune diseases.Determining counts of helper/inducer T lymphocytes can be useful in monitoring human immunodeficiency virus (HIV)-infected individuals. Individuals with HIV typically exhibit a steady decrease of helper/inducer T lymphocyte counts as the infection progresses. Suppressor/cytotoxic lymphocytes are a subset of T lymphocytes (CD3 +) that are CD8 +. CD3 + CD8 + counts are used to characterize and monitor some forms of immunodeficiency and autoimmune diseases. Suppressor/cytotoxic lymphocyte values lie outside the normal reference range in some autoimmune diseases, and in certain immune reactions such as acute graft-versus-host disease (GVHD) and transplant rejection. The CD8 + subset is elevated in many patients with either congenital or acquired immune deficiencies, such as severe combined immunodeficiency (SCID) or acquired immune

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deficiency syndrome (AIDS). The CD8 + cell population is often decreased in active systemic lupus erythematosus (SLE), but can also be increased in SLE patients undergoing steroid therapy. The Centers for Disease Control (CDC) recommends using reagent combinations containing CD3 antibodies for determining T-lymphocyte subsets in HIV-infected subjects.This kit CD4/CD8/CD3 reagent allows helper/inducer T lymphocytes to be identified and enumerated separately from contaminating CD3– and CD4 + monocytes.

Storage: Store in the dark at 2-8 °C. Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. (tech@immunostep.com).

Application: It is recommended for use in flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using5 µl/10^6 cells.

Precautions:

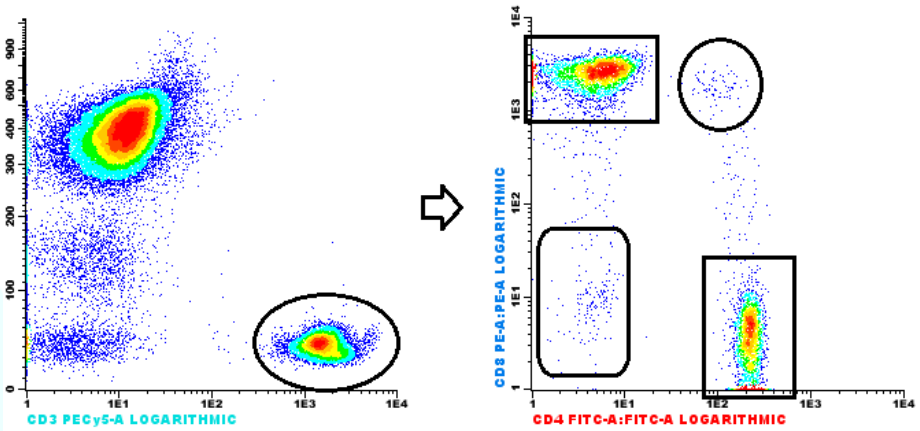
1. The device is not intended for clinical use including diagnosis, prognosis, and monitoring of a disease state, and it must not be used in conjunction with patient records or treatment.

2. This product contains sodium azide (NaN3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.

Staining Cell Surface Antigens for Flow Cytometry Protocol

1. Add 15 µL of CD4/CD8/CD3 and mix gently with a vortex mixer. The 15 µL is a guideline only; the optimal volume should be determined by the individual laboratory
2. Transfer 100 µL of anticoagulated (EDTA) blood to a 12 x 75 mm polystyrene test tube (10^6 cells).
3. Incubate in the dark at room temperature (20-25 °C) for 15 minutes or at 4 °C for 30 minutes.
4. Add Lysing Solution according to the manufacturer's directions to each sample and mix gently with a vortex mixer.
5. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant without disturbing the cell pellet and discard it leaving approximately 50 µL of fluid.
6. Add 2 mL 0.01 mol/L PBS (It better that it containing 0,5 % bovine serum albumin) and resuspend the cells. Mix well.
7. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.
8. Resuspend pellet in an appropriate fluid for flow cytometry, e.g. 0.3 mL PBS + 0,5 % BSA.

Analyse on a flow cytometer or store at 2-8 °C in the dark until analysis. Samples can be run up to 3 hours after lysis.



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The histograms are biparametric representations (Side Scatter versus Fluorescence Intensity) of a lysate normal whole blood sample gated on lymphocytes CD3+. Human peripheral blood lymphocytes were stained with three color reagents: CD4/CD8/CD3. Cells were analyzed on a FACSAria (Becton Dickinson, San Jose, CA) flow cytometer, using BD FACSDiva software.

FOR MORE INFORMATION, PLEASE VISIT OUR WEBSITE: **www.immunostep.com**

References:

1. Schmidt RE. Monoclonal antibodies for diagnosis of immunodeficiencies. *Blu*t. 1989;59:200-206.
2. Nicholson JKA. Use of flow cytometry in the evaluation and diagnosis of primary and secondary immunodeficiency diseases. *Arch Pathol Lab Me*d. 1989;113:598-605.
3. Foucar K, Goeken JA. Clinical application of immunologic techniques to the diagnosis of lymphoproliferative and immunodeficiency disorders. *Lab Me*d. 1982;13:403-413.
4. Cohen SB, Weetman AP. Activated interstitial and intraepithelial thyroid lymphocytes in autoimmune thyroid disease. *Acta Endocrino*l. 1988;119:161-166.
5. Smolen JS, Chused TM, Leiserson WM, Reeves JP, Alling D, Steinberg AD. Heterogeneity of immunoregulatory T-cell subsets in systemic lupus erythematosus: correlation with clinical features. *Am J Me*d. 1982;72:783-790.
6. Giorgi JV, Hultin LE. Lymphocyte subset alterations and immunophenotyping by flow cytometry in HIV disease.*Clin Immunol Newslet*t. 1990;10:55-61.
7. Landay A, Ohlsson-Wilhelm B, Giorgi JV. Application of flow cytometry to the study of HIV infection. *AIDS*. 1990;4:479-497.