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Product: Two Color Reagents: HLA-DR/CD3
Cat. Ref: HLADRF3PE1-50T
Reagent provided: 50 test (20µl / test)
Description: Mouse monoclonal Anti-Human HLA-DR FITC/ CD3 PE, is recommended for use in flow cytometry, is a two-color direct immunofluorescence reagent for enumerating percentages of mature human activated T lymphocytes in erythrocyte-lysed whole blood (LWB). The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ⩽0.09% sodium Azide.
Clones: GRB-1, 33-2A3
Isotypes: IgG2a, IgG2a
Flororchromes: Fluorescein isothiocyanate (*FITC*), R-Phycoerythrin (*R-PE*).

Specificity: Human lymphocytes may be divided into three major populations based on their biologic function and cell-surface antigen expression: T lymphocytes, B lymphocytes, and natural killer (NK) lymphocytes. T lymphocytes participate in antigen-specific cell-mediated immunity and regulate the secretion of immunoglobulin by B lymphocytes. T lymphocytes may also be classified based on their functional properties as helper/inducer, suppressor/cytotoxic, or activated T lymphocytes.

Clinical Aplications: Activated T lymphocytes (HLA-DR + T lymphocytes) may be elevated in states of immune activation,1 which may be caused by infection or impending transplant rejection.6 The actual cause of immune activation must be verified by additional clinical and laboratory tests.

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 using 20 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 20 µL of HLA-DR/CD3 and mix gently with a vortex mixer. The 20 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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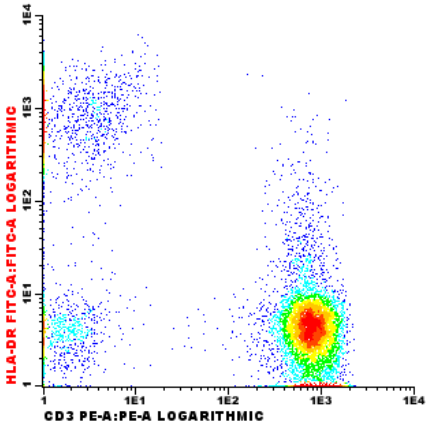

The histogram is biparametric representations (Side Scatter versus Fluorescence Intensity) of a lysate normal whole blood sample gated on Lymphocytes. Human peripheral blood lymphocytes were stained with HLA-DR/CD3. Cells were analyzed on a FACSAria (Becton Dickinson, San Jose, CA) flow cytometer, using BD FACSDiva software.

References:

1. Legendre C, Schiffrin A, Weitzner G, Colle E, Guttmann R. Two color flow cytometry analysis of activated T-lymphocyte subsets in type 1 diabetes mellitus. *Diabetes.* 1988;37:792-795. 2. Tomkinson B, Wagner D, Nelson D, Sullivan J. Activated lymphocytes during acute Epstein-Barr virus infection. *J Immunol.* 1987;139:3802-3807.
3. Bogner J, Matuschke B, Hienrich B, Schreiber M, Nerl C, Goebel F. Expansion of activated T lymphocytes (CD3 + HLA-DR +) detectable in early stages of HIV-1 infection. *Klin Wochenschr.* 1990;68:393-396.
4. Levacher M, Tallet S, Dazza M, Dournon E, Rouveix B, Pocidalo J. T activation marker evaluation in ARC patients treated with AZT: Comparison with CD4 + lymphocyte count in non-progressors and progressors towards AIDS. *Clin Exp Immuno*l. 1990;81:177-182.
5. Vanham G, Kestens L, Gigase P, et al. Evidence for circulating activated cytotoxic T cells in HIV-infected subjects before the onset of opportunistic infections. *Clin Exp Immuno*l. 1990;82:3-9.