Two Color Reagent Anti-Human Kappa light chain FITC / Anti-Human Lambda light chain PE (Rabbit F'ab 2 Polyclonal)

Fluorochrome	Reference	Test
FITC/PE	1KF3LPE2-50T	50 test

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PRODUCT DESCRIPTION

Description:
F(ab')2 Polyclonal Rabbit Anti-Human Kappa Light Chains conjugated with fluorescein isothiocyanate isomer 1 (FITC) and F(ab')2 Polyclonal Rabbit Anti-Human Lambda Light Chains, conjugated with R-phycoerythrin (R-PE) for use in flow cytometry for simultaneous detection and enumeration of kappa light chains and lambda light chains.
Clone: Polyclonal
Isotype: Rabbit F(ab')2 IgG
Reactivity: Human
Source:
Polyclonal immunoglobulin light chains of kappa type isolated from a pool of human sera for Rabbit Anti-Human Kappa Light Chains and Polyclonal immunoglobulin light chains of lambda type isolated from a pool of human sera for Rabbit Anti-Human Lambda Light Chain.
Purification: Affinity chromatography.
Composition: The conjugate is provided in aqueous buffered solution containing protein stabilizer, and $\leqslant$ 0.09% sodium Azide.

RECOMMENDED USAGE

Immunostep's kappa & lambda F'ab 2, is intended for simultaneous detection and enumeration of B lymphocytes bearing kappa light chains in peripheral blood using flow cytometry. This reagent is effective for direct immunofluorescence staining of human tissue for flow cytometric analysis using 10 µl/10_6 cells.

CLINICAL RELEVANCE

The evaluation of cell surface Kappa/Lambda expression can identify clonally restricted B lymphocyte populations and thus can aide in the diagnosis of hematologic malignancy. Several B cell disorders are associated with decreased levels of Kappa/Lambda at the cell surface.

PRINCIPLES OF THE TEST

The Rabbit F(ab)´2 Polyclonal antibody against Kappa & Lambda light chains are useful for the demonstration of cell surface kappa light chains, and, thus, for the identification of monoclonality (clonal excess) in B-cell lymphoproliferative disorders together with a panel of other antibodies. To identify these cells, the sample is incubated with the antibody and is analysed by flow cytometry.

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 ⚠

a) 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
b) Avoid microbial contamination of the reagent.
c) Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
d) Never mouth pipette.
e) In the case of contact with skin, wash in plenty of water.
f) The samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed.
g) Do not use after the expiry date indicated on the vial.
h) Deviations from the recommended procedure could invalidate the analysis results.
i) FOR *IN VITRO* DIAGNOSTIC USE.
j) For professional use only.
k) Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.

SAMPLE COLLECTION

The extraction of venous blood samples should be carried out in blood collection tubes using the appropriate anticoagulant (EDTA or heparin)[2,3]. 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 /PE	Rabbit Isotype Control	RBPLFPE-50T

- 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:

Initial Protocol to remove surface Ig:

1. Transfer 300 µL of anticoagulated (EDTA) blood to a 12 x 75 mm polystyrene test tube.
2. Add 10 mL 0.01 mol/L PBS (It better that it containing 0,5% bovine serum albumin) and resuspend the cells by using a vortex mixer.
3. Centrifuge at 540 x g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.
4. Add 10 mL 0.01 mol/L PBS (It better that it containing 0,5% bovine serum albumin) and resuspend the cells by using a vortex mixer.
5. Centrifuge at 540 x g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid. Resuspend the cell pellet in 200 µL of PBS+ 0,5% BSA.

Continue the labeling protocol of cell surface or intracellular staining protocol according to the study required.

Cell surface Ig Protocol:

1. Add 10 µL of Kappa/Lambda reagent and mix gently with a vortex mixer. The 10 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.

Intracellular staining Ig protocol:

1. For each sample, add an appropriate volume of conjugated antibody directed to the cell surface antigen of interest and the appropriate isotype control. Incubate for 15 minutes in the dark at room temperature. (This step is only necessary is you want to perform a direct immunofluorescence staining for a cell surface antigen)
2. Add 2 mL 0.01 mol/L PBS (It better that it containing 0,5 % bovine serum albumin) and resuspend the cells. Mix well.
3. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid
4. Add 100 µl of IntraCell Reagent A, (Fixative), to each tube. Mix gently. Incubate in the dark at room temperature (20-25 °C) for 15 minutes.
5. Add 2 mL 0.01 mol/L PBS (It better that it containing 0,5 % bovine serum albumin) and resuspend the cells. Mix well.
6. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.
7. Add 100 µl of IntraCell Reagent B (Permeabilization), to each tube. Add 20 µL of Kappa/Lambda reagent and mix gently with a vortex mixer.
8. Incubate for 15 minutes in the dark at room temperature.
9. Add 2 mL 0.01 mol/L PBS (It better that it containing 0,5 % bovine serum albumin) and resuspend the cells. Mix well.
10. Centrifuge at 540g for 5 minutes. Gently aspirate the supernatant and discard it leaving approximately 50 µL of fluid.
11. Resuspend pellet in an appropriate fluid for flow cytometry, e.g. 0.3 mL PBS + 0,5 % BSA.

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 polyclonal monoclonal antibody Kappa and determine the percentage of stainend cells. It is necessary to use an isotope control conjugated with the same fluorochrome, of the same type of immunoglobulin

heavy chain and concentration as that of the Kappa antibody as 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 stained peripheral blood from a healthy donor applying the protocol described in point 6:

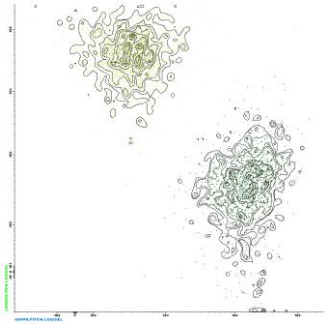

Fig. 1: Above, The histogram is biparametric representations (Side Scatter versus Fluorescence Intensity) of a lysate normal whole blood sample gated on Lymphocytes CD19+. Human peripheral blood lymphocytes were stained with KAPPA FITC, LAMBDA PE, CD19 APC and CD45 PerCP according to the Initial Protocol to remove surface Ig and Cell surface Ig Protocol. Kappa+ cells are represented by the histogram. Cells were analyzed on a FACSAria (Becton Dickinson, San Jose, CA) flow cytometer, using BD FACSDiva software.

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 un lysed 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[4].

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.

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

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