Anti- Human KAPPA (Polyclonal F'ab 2)

Fluorochrome	Reference	Test
FITC	KF3-100T	100 test
PE	KPE3-100T	100 test

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PRODUCT DESCRIPTION

Clone: Polyclonal
Isotype: Rabbit F(ab')2 IgG
Tested application: flow cytometry
Immunogen: Polyclonal immunoglobulin light chains of kappa type isolated from a pool of human sera for Rabbit Anti-Human Kappa Light Chains.
Species reactivity: Human
Storage instruction: store in the dark at 2-8 ºC
Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN_3).
Recommended usage: Immunostep's kappa $F'(ab)_2$, 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.
Presentation: Rabbit polyclonal antibody against human kappa light chains, conjugated to a fluorochrome. It is supplied in an aqueous solution containing a stabilizing protein and 0.09% sodium azide (NaN_3) as a preservative.

Fluorochrome	Supplied reagent	Concentration (mg/ml)	Volume/test
FITC	100 test	0.15	10 µL
PE	100 test	0.1	10 µL

Source: Supernatant proceeding from sera for Rabbit Anti-Human Kappa Light Chains.
Purification: Affinity chromatography.

CLINICAL RELEVANCE

This marker can be used alone or in combination with other B-cell markers for the diagnosis and monitoring of various hematologic malignancies and immunodeficiencies. Anti-kappa antibodies are essential in the immunophenotypic analysis of B-cell populations, enabling the detection of light chain expression patterns that distinguish between polyclonal and monoclonal B-cell expansions.

In healthy individuals, B cells express either kappa or lambda light chains in a balanced ratio (typically around 2:1). A significant deviation from this ratio, particularly the presence of a dominant kappa-expressing population, is indicative of B-cell clonality, a hallmark of malignancies such as chronic lymphocytic leukemia (CLL), B-cell non-Hodgkin lymphomas, and multiple myeloma.

Flow cytometric analysis using anti-kappa antibodies is also valuable in the evaluation of immunodeficiencies, where B-cell development or function may be impaired. Furthermore, it aids in distinguishing reactive polyclonal responses from neoplastic monoclonal proliferations, which is critical for accurate diagnosis and treatment planning.

ANTIGEN DETAILS

Large description: 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.[1-4]

PRINCIPLES OF THE TEST

The anti-human kappa polyclonal antibody binds specifically to kappa light chains expressed on the surface or in the cytoplasm of B cells. To identify these cells, the sample is incubated with the fluorochrome-conjugated antibody and analyzed by flow cytometry. The fluorescence intensity reflects the presence and relative abundance of kappa light chains, allowing for the characterization of B-cell populations and the assessment of clonality.

APPROPRIATE STORAGE AND HANDLING CONDITIONS

Store in the dark, refrigerated between 2 ºC and 8 ºC. DO NOT FREEZE. The antibody is stable until the expiry date stated on the vial label if kept at 2ºC-8ºC. 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)[7,8]. 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.
Please, refer to www.immunostep.com technical support for more information.

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.

1. MATERIALS REQUIRED BUT NOT PROVIDED

- 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 (Ref. RBC-10X)
- Flow cytometer equipped with laser and appropriate fluorochrome filters
- Vortex Agitator

2. SAMPLE PREPARATION: PROCEDURE WITH PRE-WASH

1. Pipette 300 µL of sample into a 10 mL Falcon tube.
2. Add 6 mL of washing buffer and mix well, preferably by gently vortexing.
3. Fill the tube up to 10 mL by adding an additional 4 mL of washing buffer.
4. Mix well again by gently vortexing.
5. Centrifuge at 540 g for 5 minutes.
6. Carefully discard the supernatant using a Pasteur pipette or vacuum system, avoiding disturbance of the cell pellet.
7. Resuspend the pellet gently.
8. Repeat steps 2 to 7 two more times (for a total of three washing steps).
9. After the final wash, resuspend the cell pellet in 300 µL of washing buffer.
10. Continue with the standard protocol for staining of surface or intracellular antibodies.
11. Note 1: For small volume samples (e.g., cerebrospinal fluid or vitreous aspirates), centrifuge the entire volume at 540 g for 5 minutes, discard the supernatant, and resuspend in 300 µL of washing buffer before proceeding.

Acquire on a flow cytometer or store in the dark at 2ºC -8ºC until the analysis is carried out. Samples should be acquired within the 3 hour after lysis.

3. FLOW CYTOMETRY ANALYSIS

Collect the fluorescence signal associated with the antibody and determine the percentage of positively labeled cells. An isotype control conjugated with the same fluorochrome, immunoglobulin isotype, and concentration should be used to assess and correct for non-specific binding to lymphocytes (see "Materials Required but Not Provided"). Define an analysis gate to exclude background fluorescence and accurately identify positively labeled cell populations.

4. 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.

5. 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[4,5,6].

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.

6. CHARACTERISTICS

SPECIFICITY

The rabbit polyclonal antibody against human kappa light chains ($F(ab')_2$ fragment) specifically recognizes the kappa light chain of immunoglobulins, a 25 kDa polypeptide encoded by the IGK gene on chromosome 2[6]. These light chains are expressed on the surface of approximately two-thirds of mature B lymphocytes in peripheral blood, as well as on a subset of immature B cells in bone marrow. The antibody targets both surface and cytoplasmic kappa chains, allowing for comprehensive immunophenotypic analysis of B-cell populations[7].

To evaluate analytical specificity, two complementary experimental approaches were conducted:

1. Cell Population Profiling

Peripheral blood samples from healthy donors were stained using a multicolor flow cytometry panel including the test antibody (Kappa PE) and a reference antibody. The expression of kappa light chains was analyzed across lymphoid and myeloid subsets. The test antibody showed consistent and specific binding to $CD19^+$ B cells, with a mean positivity of 52.4% and low variability (CV = 4.93%). Subsets such as naïve ($CD27^-/IgD^+$), memory ($CD27^+/IgD^-$), and natural effector B cells ($CD27^+/IgD^+$) were all positively stained, confirming broad reactivity within the B-cell compartment. Minimal or no staining was observed in T cells ($CD3^+$), NK cells, and granulocytes, supporting the antibody's specificity.

2. Blocking Assay

To assess epitope specificity and potential cross-reactivity, a blocking experiment was performed using a reference polyclonal anti-kappa $F(ab')_2$ antibody. Samples were co-stained with both the test and reference antibodies in reciprocal combinations. No complete blocking was observed, confirming that the test antibody binds to distinct or non-overlapping epitopes within the kappa light chain. This is consistent with the expected behavior of polyclonal antibodies, which recognize multiple epitopes.

These results validate the analytical specificity of the antibody for kappa light chains and support its use in flow cytometric applications for B-cell characterization, clonality assessment, and immunodeficiency evaluation.

SENSIBILITY

The analytical sensitivity of the anti-kappa antibody was evaluated using a serial dilution model with Daudi ($kappa^+$) and Namalwa ($lambda^+$) cell lines. Each dilution was adjusted to a final concentration of 1×10^5 cells per tube, and analyzed in triplicate across nine concentration levels, following the statistical approach described in the CLSI guideline "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline", Appendix C.

Marker	R^2	LoD	LoB
FITC	0.996	1.72%	1.06%
PE	0.999	8.69%	7.64%

The mean fluorescence intensity (MFI) was calculated for each dilution level. The results demonstrated a strong linear relationship between the dilution level and the MFI, with a coefficient of determination (R^2) of 0.99, indicating excellent linearity across the tested range.

These findings confirm that the antibody provides a reliable and proportional signal across a wide range of antigen concentrations, supporting its use in quantitative flow cytometric applications.

PRECISION

Repeatability
Twelve replicates per sample were analyzed to assess intra-assay variability and repeatability. The standard deviation (SD) and coefficient of variation (CV%) for each sample were:

Marker	Mean MFI	Repeatability SD	Repeatability %CV	Within-site SD	Within-site %CV
FITC	59.98	0.38	0.6	0.37	0.6
PE	54.09	2.23	4.12	1.75	3.24

These results demonstrate excellent repeatability and onsistent performance across different reagent lots and testing conditions within the same laboratory under controlled laboratory conditions.

Between-Laboratory Precision

The mean of five replicates per day was calculated and compared across lots. The variability between lots was negligible, with:

Marker	Between-lab SD	Between-lab %CV	Between-lot SD	Between-lot %CV
FITC	0.24	0.40	0.62	1
PE	2.62	4.84	2.53	4.68

This confirms the consistency of the antibody performance across different production batches.

Reproducibility
Reproducibility was assessed using stabilized samples (Transfix or Streck) processed at three independent laboratories. Each site performed five replicates per day over five non-consecutive days.

Reproducibility was assessed using stabilized samples processed at multiple laboratories. The inter-day and inter-laboratory variability were as follows:

Marker	Reproducibility SD	Reproducibility %CV
FITC	1.01	1.7
PE	4.04	7.47

These results confirm that the antibody provides consistent and reproducible staining across different laboratories and testing conditions.

7. WARRANTY

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8. BIBLIOGRAPHY

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