Anti-Human KAPPA/LAMBDA (Polyclonal F(ab')$_2$)

Fluorochrome	Reference	Test
FITC/PE	**1KF3LPE2-50T**	**50 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 and Lambda type isolated from a pool of human sera.
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/Lambda, is intended for simultaneous detection and enumeration of B lymphocytes bearing lambda 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: Liquid.
Rabbit polyclonal antibody targeting human kappa and lambda light chains, conjugated to FITC and PE fluorochromes, respectively. 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 PE	50 test	0.15 0.1	10 µL

Source: the antibodies are sourced from two purified sera obtained from rabbits; one rabbit was immunized with human lambda light chains, while the other was immunized with human kappa light chains..
Purification: Affinity chromatography.

CLINICAL RELEVANCE

Anti-Human KAPPA/LAMBDA which includes both anti-kappa and anti-lambda antibodies, is essential for the immunophenotypic characterization of B-cell populations and plays a key role in the diagnosis and monitoring of hematologic malignancies and immunodeficiencies. The analysis of light chain expression patterns enables the distinction between polyclonal and monoclonal B-cell expansions.

In healthy individuals, B lymphocytes express either kappa or lambda immunoglobulin light chains, maintaining a relatively constant kappa:lambda ratio—typically around 2:1. A significant deviation from this ratio, especially the presence of a dominant population expressing only kappa or only lambda chains, is indicative of light chain restriction, a hallmark of B-cell clonality. This finding is commonly associated with disorders such as chronic lymphocytic leukemia (CLL), B-cell non-Hodgkin lymphomas, and multiple myeloma.

Flow cytometric analysis using anti-kappa and anti-lambda antibodies facilitates the rapid identification of clonal B-cell populations, supporting differential diagnosis between reactive polyclonal processes and neoplastic monoclonal proliferations. This distinction is crucial for accurate diagnosis, prognosis, and guiding therapeutic decisions.

Moreover, the combined assessment of light chain expression is valuable in the evaluation of primary and secondary immunodeficiencies, where disturbances in B-cell development or function may alter the normal distribution of light chains.

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/LAMBDA polyclonal antibody binds specifically to lambda 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 lambda 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.

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 attributed to antibody and determine the percentage of positive cells. It is necessary to use an isotope control conjugated with the same fluorochrome, of the same type of immunoglobulin and concentration, so 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 labelled cells.

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

Rabbit polyclonal antibodies against human lambda light chains specifically recognize the lambda subunit of immunoglobulins, which is expressed on the surface of approximately one-third of mature B lymphocytes in peripheral blood, as well as on a subset of immature B cells in bone marrow.

1. Cell Population Profiling

To evaluate analytical specificity, a comparative study was conducted using a multicolor flow cytometry panel including the test antibody (Immunostep) and a reference antibody. Peripheral blood samples from healthy donors were analyzed to assess marker expression across various leukocyte subsets.

The test antibody showed strong and specific staining of $CD19^+$ B cells, with a mean positivity of 30.9% and low variability (CV = 3.11%). Subsets such as naïve ($CD27^-/IgD^+$) and memory B cells ($CD27^+/IgD^-$) were also clearly identified, confirming the antibody's ability to detect lambda light chains across different stages of B-cell maturation. Minimal or no staining was observed in T cells, NK cells, and granulocytes, supporting the antibody's specificity.

Notably, plasmacytoid dendritic cells ($CD123^+$/HLA-DR^+) and monocyte-derived dendritic cells ($CD16^-/CD14^-$) also showed high positivity, consistent with known expression patterns of surface immunoglobulins in certain antigen-presenting cell subsets.

2. Blocking Assay

To further confirm specificity, a blocking experiment was performed using the Dako polyclonal anti-lambda $F(ab')_2$ antibody. Samples were co-stained with both the test and reference antibodies in reciprocal combinations. No complete blocking was observed, indicating that the antibodies bind to distinct or non-overlapping epitopes, as expected for polyclonal reagents.

These results confirm the analytical specificity of the anti-lambda antibody for flow cytometric applications, supporting its use in the identification of B-cell subsets, assessment of clonality, and evaluation of immunodeficiencies.

SENSIBILITY

The analytical sensitivity of the anti-lambda PE antibody was evaluated using a serial dilution model with Daudi ($lambda^+$) and Namalwa ($lambda^+$) cell lines. Each dilution was adjusted to a final concentration of 1×10^5 cells per tube, and analyzed in duplicate 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.

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 0.978 and 0.996, indicating excellent linearity across the tested range.

Marker	R^2	LoB %	LoD %	LoQ %
KAPPA FITC	0.978	1.06	1.72	6.41
LAMBDA PE	0.996	0.647	0.656	4.2

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. The Limit of Blank (LoB), the Limit of Detection (LoD) and the Limit of Quantification (LoQ) confirm the assay's high sensitivity and reliable quantification at low concentration levels.

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

Precision was assessed following the CLSI EP05-A3 guideline using a 3×3×2×2 study design, which included three levels of samples (low, medium, high), three independent reagent lots, two Cytek Aurora flow cytometers, and two replicates per condition. The study did not include variability across days. Data acquisition was performed on the Cytek Aurora instruments, and analysis was conducted using the Infinicyt™ software (BD Biosciences). This design enabled the evaluation of both repeatability (within-run precision) and intermediate precision across instruments and reagent lots under controlled laboratory conditions.

Repeatability

To assess intra-assay variability and repeatability, three replicates per sample level were analyzed across three reagent lots and two Cytek Aurora flow cytometers, resulting in a total of 54 measurements per sample level. The standard deviation (SD) and coefficient of variation (CV%) were calculated for each condition using data processed in Infinicyt™ (BD Biosciences), providing a robust estimate of repeatability under controlled laboratory settings.

Marker	Repeatability SD	Repeatability %CV	Within-site SD	Within-site %CV
FITC	0.38	0.6	0.41	0.7
PE	1.59	4.2	2.56	5.2

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

Between-Laboratory Precision

Between-laboratory precision was evaluated by analyzing three replicates per sample level. Although the study did not include day-to-day variability, the use of two independent instruments and multiple reagent lots allowed for the estimation of intermediate precision under controlled inter-laboratory conditions. Data were analyzed and the standard deviation (SD) and coefficient of variation (CV%) were calculated to characterize the variability attributable to instrumentation and reagent lot differences. The variability between lab and lot was negligible, with:

Marker	Between-lab SD	Between-lab %CV	Between-lot SD	Between-lot %CV
FITC	0.24	0.40	0.62	1.0
PE	1.55	3.99	2.43	3.7

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

Reproducibility

Reproducibility was assessed by evaluating three replicates per sample level across three reagent lots and two Cytek Aurora flow cytometers, totaling 36 measurements. Although the study did not include inter-day or inter-operator variability, the use of multiple instruments and reagent lots across laboratories provided a meaningful estimate of reproducibility under controlled multi-laboratory conditions. Data analysis was performed using Infinicyt™ (BD Biosciences), and the standard deviation (SD) and coefficient of variation (CV%) were calculated to quantify the overall variability attributable to laboratory and equipment differences.

Reproducibility was assessed using stabilized samples processed at multiple laboratories. The reproducibility were as follows:

Marker	Reproducibility SD	Reproducibility %CV
FITC	1.01	1.7
PE	4.48	10.2

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

7. 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's sole liability is limited to either the replacement of the products or refund of the purchase price.

8. BIBLIOGRAPHY

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9. MANUFACTURED BY

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9. SURVEILLANCE AND NOTIFICATION

In accordance with Annex I, Section 20.4.1(n) of Regulation (EU) 2017/746, the user is obliged to report any serious incident related to the use of the product.

- **To the Manufacturer:** Please contact our Surveillance Department at vigilancia@immunostep.com
- **To the Competent Authority:** Report via the official channels of the Member State.

10. EXPLANATION OF SYMBOLS

CE	CE labeling
IVD	*In vitro* diagnostic device
	Manufacturer
	Pay attention to