

**Product:** Three Color Reagent Anti-Human Lambda light chain FITC / Kappa light chain PE/ CD19 PE-Cyanine 5.

**Cat. Ref:** LF2KPE319PC4-50

**Reagent provided:** 50 TEST (20µl / test)

**Description:** F(ab')<sub>2</sub> Polyclonal Rabbit Anti-Human Lambda Light Chains conjugated with fluorescein isothiocyanate isomer 1 (FITC), F(ab')<sub>2</sub> Polyclonal Rabbit Anti-Human Kappa Light Chains conjugated with R-phycoerythrin (R-PE) and PE-Cyanine 5 Anti-Human CD19 for use in flow cytometry for simultaneous detection and enumeration of kappa light chains and lambda light chains. The conjugate is provided in aqueous buffered solution containing protein stabilizer, and ≤0.09% sodium Azide. Each vial contains 50 tests (20 µL of conjugate for up to 10<sup>6</sup> leucocytes from normal human peripheral blood).

**Host:** Rabbit (Lambda and Kappa) and Mouse (CD19)

**Clone:** Polyclonal (Lambda and Kappa) and HIB19 (CD19)

**Isotypes:** Rabbit F(ab')<sub>2</sub> IgG (Lambda and Kappa) and Mouse IgG1 kappa (CD19)

**Immunogen:** Polyclonal immunoglobulin light chains of Lambda type isolated from a pool of human sera for Rabbit Anti-Human Lambda Light Chains and Polyclonal immunoglobulin light chains of Kappa type isolated from a pool of human sera for Rabbit Anti-Human Kappa Light Chain.

**Fluorochromes:** Fluorescein isothiocyanate (FITC), R-Phycoerythrin (R-PE) and PE-Cyanine. PE-Cyanine5 is a tandem dye formed by the combination of PE and Cyanine 5.

**Specificity:** Lambda and Kappa polyclonal have been produced from F(ab')<sub>2</sub> fragments of affinity-isolated polyclonal rabbit antibodies. CD19 was purified from Hybridome supernatant.

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

**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](mailto: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<sup>6</sup> 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 (NaN<sub>3</sub>), 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.

**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:

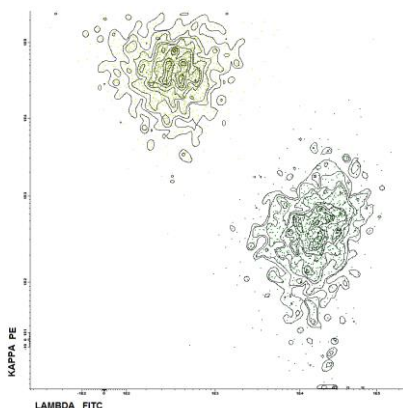
1. Add 20  $\mu\text{L}$  of Lambda/Kappa/CD19 reagent and mix gently with a vortex mixer. The 20  $\mu\text{L}$  is a guideline only; the optimal volume should be determined by the individual laboratory.
2. Transfer 100  $\mu\text{L}$  of anticoagulated (EDTA) blood to a 12 x 75 mm polystyrene test tube ( $10^6$  cells).
3. Incubate in the dark at room temperature (20-25  $^{\circ}\text{C}$ ) for 15 minutes or at 4  $^{\circ}\text{C}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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  $^{\circ}\text{C}$  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  $\mu\text{L}$  of fluid
4. Add 100  $\mu\text{L}$  of IntraCell Reagent A, (Fixative), to each tube. Mix gently. Incubate in the dark at room temperature (20-25  $^{\circ}\text{C}$ ) 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  $\mu\text{L}$  of fluid.
7. Add 100  $\mu\text{L}$  of IntraCell Reagent B (Permeabilization), to each tube. Add 20  $\mu\text{L}$  of Lambda/Kappa /CD19 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  $\mu\text{L}$  of fluid.
11. 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  $^{\circ}\text{C}$  in the dark until analysis. Samples can be run up to 3 hours after lysis.



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 LAMBDA FITC, KAPPA PE, CD19 PE-Cyanine 5 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.

*FOR MORE INFORMATION, PLEASE VISIT OUR WEBSITE: [www.immunostep.com](http://www.immunostep.com)*

**References:**

1. Picker, L.J., et al., 1987, Am.J.Path., 128. 1811. Johnson A, Olofsson T. Flow cytometric clonal excess analysis of peripheral blood, routine handling, and pitfalls in interpretation. Cytometry 1993; 14: 188- 95.
2. Cartron G, Linassier C, Bremond JL, Desablens B, Georget MT, Fimbel B, et al. CD5 negative B- cell chronic lymphocytic leukemia: clinical and biological features of 42 cases. Leuk Lymphoma 1998; 31: 209- 16.
3. Gandini D, Lanza F, Latorraca A, Levato F, Del Senno L, Castoldi G. Immunophenotypic and genotypic characterization of B- cell chronic lymphocytic leukemia patients from Northern Italy. Haematologica 1993; 78: 18- 24.
4. Braylan RC, Orfao A, Borowitz MJ, Davis BH. Optimal number of reagents required to evaluate hematolymphoid neoplasias: results of an international consensus meeting. Cytometry 2001; 46: 23-7.

**\*Note: For research use only. Not for diagnostic use. Not for resale. Immunostep will not be held responsible for patent infringement or other violations that may occur with the use of our products.**