Polyclonal Anti Human Kappa Light Chains-APC Goat F(ab')$_2$

Ref: CYT-KAPPAP

For Research Use Only

INTENDED USE

Polyclonal Anti-Kappa Light Chains labelled with allophycocyanine (APC) is an antibody designed for use as a direct immunofluorescence reagent in the identification and enumeration of cells which express Kappa immunoglobulin Light Chains by flow cytometry. Antibodies to Kappa Light Chains are useful for the identification of clonal excess in B-cell lymphoproliferative disorders together with a panel of other antibodies [(1)].

Anti-Kappa Light Chains reacts with free Kappa chains as well as Kappa chains in intact immunoglobulin molecules.

SUMMARY AND EXPLANATION

Flow Cytometry is a powerful tool for the analytical and quantitative characterization of cells which provides rapid, quantitative and multiparametric analysis of heterogeneous cell populations on a cell-by-cell basis. Flow cytometry is performed on cells in liquid suspension that have been incubated with fluorescently-labeled antibodies directed against specific cellular proteins. The relative fluorescence intensity of the positive cells indicates the amount of antibody bound to specific binding sites on the cells, and therefore provides a relative measure of antigen expression.

Human lymphocytes may be classified in three main populations according to their biological function and their cell surface antigen expression: T lymphocytes, B lymphocytes and natural killer cells (NK). B lymphocytes are the producers of antibodies and mediate humoral immunity particularly effective against toxins, whole bacteria, and free viruses.

Most B cells, with the exception of pre-B progenitors, pre B cells, and mature plasma cells, express immunoglobulin on their surface. Each cell expresses only one Light Chain type. In normal peripheral blood and lymph nodes, there is a mixture of Kappa positive and Lambda positive cells, with two-thirds of the cells expressing Kappa and one-third expressing Lambda. Since lymphoid neoplasms are usually clonal expansions of a single cell, malignant cells uniformly express the same Light Chain isotype. Neoplastic B cell lymphoprolipherative disorders can frequently be suspected on the basis of the demonstration of a marker predominance of cells expressing a single Light Chain type [(1-4).]

PRINCIPLES OF THE PROCEDURE

Flow cytometry (FC) is an innovative technology by means of which different cell characteristics are simultaneously analyzed on a single cell basis. This is achieved by means of hydrodynamic focusing of cells that pass aligned one by one in front of a set of light detectors; at the same time they are illuminated by a laser beam. The interaction of the cells with the laser beam generates signals of two different kinds: those generated by dispersed light (FSC/SSC), which mainly reflects the size of the cell and its internal complexity, and those related to the emission of light by the fluorochromes present in the cell. These signals become electric impulses which are amplified and registered as digital signals to be processed by a computer.

When the reagent is added to the sample, the fluorochrome-labelled antibody presents in the reagent binds specifically to the antigens they are directed against, allowing the detection by FC of the cell populations carried by the antigen.

The erythrocyte population, which could hinder the detection of the target population, is eliminated by the use of an erythrocyte lysing solution containing fixatives previous to acquire the sample on the cytometer.

The Kappa immunoglobulin Light Chains count is generally expressed as a percentage of B cells present in the sample which can be determined by FC based on its positive expression of CD19. Because each flow cytometer has different operating characteristics each laboratory must determine its optimal operating procedure.

REAGENT COMPOSITION

Purified polyclonal antibody Anti-Kappa Light Chains, Goat F(ab')$_2$, conjugated with allophycocyanine (APC), supplied in phosphate buffered saline with 0.1% sodium azide.

Amount per 0.5 ml vial: 100 tests (5 µl/ test)

Reagents are not considered sterile.

STORAGE CONDITIONS

The reagent is stable until the expiration date shown on the label, when stored at 2-8º C. The reagent should not be frozen or exposed to direct light during storage or during incubation with cells. Keep the reagent vial dry. Once opened, the vial must be stored in a vertical position to avoid any possible spillage.

WARNINGS AND RECOMMENDATIONS

1. For research use only.
2. This product is supplied ready to use. If it is altered by dilution or addition of other components, such conditions must be validated by the user.
3. The reagent is stable until the expiration date shown on the label if it is properly stored. Do no use after the expiration date shown on the label. If the reagents are stored in conditions different from those recommended, such conditions must be validated by the user.
4. Alteration in the appearance of the reagent, such as the precipitation or discoloration indicates instability or deterioration. In such cases, the reagent should not be used.
5. It contains 0.1% sodium azide (CAS-Nr. 26628-22-8) as a preservative, but even so care should be taken to avoid microbial contamination of reagent or incorrect results may occur.
    - Sodium azide (NaN_3) is harmful if swallowed (R22), if swallowed, seek medical advice immediately and show this container or label (S46).
    - Wear suitable protecting clothing (S36).
    - Contact with acids liberates very toxic gas (R32).
    - Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in metal drains where explosive conditions may develop.

6. All patient specimens and materials with which they come into contact are considered biohazards and should be handled as if capable of transmitting infection [5], and disposed according to the legal precautions established for this type of product. Also recommended is handling of the product with appropriate protective gloves and clothing, and its use by personnel sufficiently qualified for the procedures described. Avoid contact of samples with skin and mucous membranes. After contact with skin, wash immediately with plenty of water.
7. Use of the reagent with incubation times or temperatures different from those recommended may cause erroneous results. Any such changes must be validated by the user.

PROCEDURE

Material included

Polyclonal Anti-Kappa Light Chains-APC sufficient for 100 determinations (5 µl/test).

Material required but not included

- Flow cytometer equipped with 633 red laser and appropriate computer hardware and software.
- Test tubes suitable for obtaining samples in the flow cytometer used. Usually tubes with a rounded bottom for 6 mL, 12x 75 mm are used.
- 10 mL tubes to perform a bulk wash procedure.
- Automatic pipette (100µL) and tips.
- Micropipette with tips.
- Chronometer.
- Vortex Mixer.
- Centrifuge.
- Pasteur pipette or vacuum system.
- Isotype control reagent.
- Erythrocyte lysing solution.
- Wash buffer as phosphate buffered saline (PBS) + 0.5% Bovine Serum Albumin (BSA)

Preparation

Whole blood sample must be taken aseptically by means of a venipuncture [6, 7] in a sterilized tube for blood collection containing an appropriate anticoagulant (use of EDTA is recommended). Store the blood samples at 18-22ºC until they are to be tested. It is advisable to test blood samples within the 24 hours after their extraction. Hemolyzed samples or samples with suspended cell aggregates should be rejected.

1. **As this procedure for sample staining includes surface membrane (Sm) immunoglobulins (Ig) staining, sample to study must be washed twice to remove the soluble serum proteins (steps 1a-1j). Be careful with volumes after discarding supernatants.**
    a. Pipette 300 µL of sample into a 10 mL tube. For small samples (i.e. CSF, vitreous aspirates) spin down the total volume (5 min at 540 g), discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, and resuspend in 300 µL of PBS + 0.5% of Bovine Serum Albumin (BSA).
    b. Add 10 mL filtered PBS + 0.5% BSA.
    c. Mix well.
    d. Centrifuge for 5 min at 540 g.
    e. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet.
    f. Add 10 mL PBS + 0.5% of BSA to the cell pellet.
    g. Mix well.
    h. Centrifuge for 5 min at 540 g.
    i. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet.
    j. Resuspend the cell pellet in 200 µL of PBS + 0.5% BSA.

2. Use 50 µL of this sample in a new tube and add 5 µL of Polyclonal Anti-Kappa Light Chains-APC. Additionally add the appropriate volume of other antibodies to include in the surface staining. To evaluate the non-specific binding of the antibody, an appropriated isotype control tube can be prepared.
3. Mix well.
4. Incubate for 15 min at room temperature (RT) protected from light.
5. Add 2 mL of an erythrocyte lysing solution containing fixatives.
6. Mix well.
7. Incubate for 10 min at room temperature protected from light.
8. Centrifuge for 5 min at 540g.
9. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leave approximately 50 µL residual volume in each tube.
10. Wash by adding 2 mL of PBS + 0.5% of BSA to the cell pellet.
11. Mix well.
12. Centrifuge for 5 min at 540g.
13. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leave approximately 50 µL residual volume in each tube.
14. Resuspend the cell pellet in 200 µL PBS + 0.5% of BSA
15. Acquire directly on the flow cytometer within the first hours after finishing the sample preparation. If the samples are not acquired immediately after preparation, they should be stored in the dark at 4-8ºC.

Flow cytometry analysis

Check that cytometer is correctly aligned and standardized for light dispersion and fluorescent intensity, and that the right color compensation has been set following the instructions of the cytometer manufacturer.
The Kappa immunoglobulin Light Chains count is generally expressed as a percentage of B cells present in the sample.

LIMITATIONS

- Blood samples should be stored at 18-22ºC and be tested within the 24 hours after they are obtained.
- It is advisable to acquire stained samples on the cytometer as soon as possible to optimize the results. Nonviable cells may stain nonspecifically. Prolonged exposure of whole blood samples to lytic reagents may cause white cell destruction and loss of cells from the target population.
- When using whole blood procedures, all red blood cells may not lyse under following conditions: nucleated red blood cells, abnormal protein concentration or hemoglobinopathies. This may cause falsely decreased results due to unlysed red blood cells being counted as leukocytes.
- Results obtained by flow cytometry may be erroneous if the cytometer laser is misaligned or the gates are improperly set.
- Each laboratory should establish a normal range for B-cells bearing Kappa Light Chains using its own test conditions.
- Certain patients may present special problems due to altered or very low number of certain cellular population.
- Cells separated from whole blood by means of density gradients may not have the same relative concentrations of cells as unseparated blood. This may be relatively insignificant for samples from individuals with normal white blood cell counts. In leukopenic patients, the selective loss of specific subsets may affect the accuracy of the determination.
- It is important to understand the normal pattern of expression of this antigen and its relation to the expression of other relevant antigens to carry out an adequate analysis. [8]
- Abnormal states of health are not always represented by abnormal percentages of certain leukocyte populations. An individual who may be in an abnormal state of health may show the same leukocyte percentages as a healthy person.

EXPECTED VALUES

Each laboratory should establish its own normal reference ranges for B cell (CD19+) counts, since such values may be influenced by age, sex and race [9]. In normal peripheral blood and lymph nodes, there is a mixture of Kappa positive and Lambda positive B cells, with two-thirds of the B cells expressing Kappa and one-third expressing Lambda [10].

QUALITY CONTROL

- To obtain optimum results it is advisable to verify the precision of pipettes and that the cytometer is correctly calibrated.
- In multicolor panels fluorochromes emit in different wavelengths but show a certain spectral overlapping which must be corrected by means of electronic compensation. The optimum levels of compensation can be established by analysis in a dot-plot diagram of cells from healthy individuals stained with mutually exclusive monoclonal antibodies conjugated with the fluorochromes to be used in the test.
- To evaluate the non-specific binding of the antibody, an appropriated isotype control tube can be prepared.

REFERENCES

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WARRANTY

This product is warranted only to conform to the quantity and contents stated on the label. There are no warranties that extend beyond the description on the label of the product. Cytognos's sole liability is limited to either replacement of the product or refund of the purchase price.

EXPLANATION OF SYMBOLS

	Use by (YYYY-MM)
	Storage temperature limitation
	Consult instructions for use
RUO	For research use only
	Batch code
	Catalogue number
	Manufacter

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