LC12

Lymphoclonal 12

Pacific Blue™	OC515	FITC	PE	PerCP-Cyanine 5.5	PE-Cyanine7	APC	APC-C750
CD20+ CD4	CD45	CD8+ SmIgλ	CD56+ SmIgK	CD5	CD19	CD3+ CD10	CD38

Ref: CYT-LC12

For Research use only

LC12 VIALS ARE A LYOPHILIZED PRODUCT. READ CAREFULLY THE FOLLOWING INSTRUCTIONS FOR RECONSTITUTION:

The lyophilized LC12 kit preserves the stability of the pre-mixed combination of antibodies. Reconstitute each lyophilized vial containing the pre-mixed combinations with **300 µl of distilled water**. Unused volume of reconstituted vial is stable during one month from date of reconstitution if it is stored in the dark at 2-8º C.

INTENDED USE

Lymphoclonal 12 Tube (LC12) is a kit with 12 conjugated antibodies designed for the detection of aberrant mature lymphocyte populations of B, T and NK lineage by flow cytometry. This 8-color combination can be used for evaluation of several suspected clinical conditions, such as lymphocytosis, lymph node enlargement, splenomegaly, monoclonal serum components, unexplained cytopenias, etc [1]. This reagent must be used by flow cytometry qualified personal.

SUMMARY AND EXPLANATION

Detection of phenotypically aberrant and clonal mature lymphocytes is the diagnostic hallmark of chronic lymphoproliferative disorders (CLPD). Clonogenic events lead to the expansion and accumulation of mature-appearing lymphocytes, which carry a proliferative and/or survival advantage over their normal counterparts.

Flow Cytometry is a powerful tool 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.

LC12 kit recognizes by flow cytometry the antigens CD45, CD3, CD56, CD4, CD8, CD5, CD20, CD19, CD10, CD38, kappa and lambda light chains expressed by different lymphocyte subsets and plasma cells, and can therefore be used in the immunophenotypic characterization studies of lymphocytes and plasma cells. These studies are widely applied in the characterization and follow-up of different hematological malignancies [2-5].

PRINCIPLES OF THE PROCEDURE

Multiparameter flow cytometry 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 reagents are added to the sample, the mixture of fluorochrome-labeled antibodies bind specifically to the antigens they are directed against, allowing the detection by flow cytometry of the different lymphoid subsets.

The mature erythrocyte population, which could hinder the detection of the target population, is eliminated by the use of a red blood cell lysing solution previous to acquire the sample on the cytometer.

REAGENT COMPOSITION

LC12 kit contains sufficient volume for 25 tests distributed in lyophilized vials of 5 tests each. LC12 kit includes a combination of Surface Membrane (Sm) staining antibodies identified as follows:

5 vials of 5 tests each for surface staining with the following lyophilized pre-mixed combination of antibodies:

- Anti-human CD4/20-Pacific Blue™ antibody, clone: RPA-T4/ 2H7, isotype: IgG1/IgG2b
- Anti-human CD45-OC515 antibody, clone: GA90, isotype: IgG2a.
- Anti-human CD8/IgmΛ-FITC antibody, clone: UCHT-4/Poly, isotype: IgG2a/----.
- Anti-human CD56/IgmK-PE antibody, clone: C5.9/Poly, isotype: IgG2b/----.
- Anti-human CD5-PerCP-Cyanine 5.5 antibody, clone: UCHT-2, isotype: IgG1.
- Anti-human CD19-PE-Cyanine7 antibody clone: 19-1, isotype IgG1.
- Anti-human CD3/CD10-APC antibody, clone: 33-2A3/MEM-78, isotype: IgG2a/IgG1.
- Anti-human CD38-APC-C750 antibody, clone: LD38, isotype: IgG1.

Fluorochrome	Pacific Blue™		OC515	FITC		PE		PerCP-Cyanine5.5	PE-Cyanine7	APC		APC-C750
Marker	CD20	CD4	CD45	CD8	SmIgΛ	CD56	SmIgK	CD5	CD19	CD3	CD10	CD38
Clone	2H7	RPA-T4	GA90	UCHT-4	Polyclonal	C5.9	Polyclonal	UCHT-2	19-1	33-2A3	MEM-78	LD38
Isotype	IgG2b	IgG1	IgG2a	IgG2a		IgG2b		IgG1	IgG1	IgG2a	IgG1	IgG1
Reactivity	B cells	T cell subset	Leukocytes	T cell subset	Lambda Ig light chain	NK cells	Kappa Ig light chain	T cells	B cells	Mature T cells	B Prec. and Granulocytes	Plasma cells

All components contain ≤0,09% (m/v) sodium azide (NaN_3). Reagents are not considered sterile.

STORAGE CONDITIONS

LC12 kit is stable until the expiration date shown on the label, when stored at 2-8º C. The expiration date applies to the lyophilized product. After reconstitution vials with the pre-mixed combination are stable for one month when stored at 2-8º protected from light. Components should not be frozen or exposed to direct light during storage or during incubation with cells. Keep vials in a dry place. Once opened, vials must be stored in a vertical position to avoid any possible spillage.

RECONSTITUTION:

The lyophilized LC12 kit preserves the stability of the pre-mixed combination of antibodies. Reconstitute each lyophilized vial containing the pre-mixed combinations with 300µl of distilled water. Unused volume of reconstituted vial is stable during one month from date of reconstitution if it is stored at 2-8º C.

WARNINGS AND RECOMMENDATIONS

1. For research use only
2. If components of this kit are altered by addition of other components, such conditions must be validated by the user.
3. The kit is stable until the expiration date shown on the label if it is properly stored. Do not 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 reagents, such as the precipitation indicates instability or deterioration. In such cases, the reagents should not be used.
5. It contains 0,09% (m/v) sodium azide (CAS-No. 26628-22-8) as a preservative, but even so care should be taken to avoid microbial contamination of reagent or incorrect results may occur.

   Indication(s) of danger:

   H302 Harmful if swallowed

   Safety advice:

   P264 Wash thoroughly after handling.
   P270 Do not eat, drink or smoke when using this product.
   P301+P312 If swallowed, call a POISON CENTER or doctor/physician if you feel unwell.
   P301+P330 If swallowed, rinse mouth.
   P501Dispose of contents/container in accordance with local/regional/national/international regulation.
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 [7], 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 reagents with incubation times or temperatures different from those recommended may cause erroneous results. Any such changes must be validated by the user.
8. Any serious incident relating to the product must be reported to Cytognos SL as well as the competent professional authority of the Member State in which the user is established.

PROCEDURE

Material included

LC12 is sufficient for 25 determinations. It includes the following reagents:

- **5 Lyophilized vials** with a pre-mixed combination of 12 conjugated antibodies.
- Additionally, CD45-OC515, CD19-PE-Cyanine7 and CD38-APC-C750 **compensation vials** for 5 tests (5µl/test). These compensations vials are in liquid format and ready to use. Compensation requirements for OC515, PE-Cyanine7 and APC-C750 are similar to Pacific Orange™ , PECy7 and APC-H7, respectively.

Material required but not included

- Test tubes suitable for running samples in the flow cytometer used. Usually tubes with a rounded bottom for 6 ml, 12x75 mm are used.
- Automatic pipettes and tips.
- Micropipette with tips.
- Vortex Mixer.
- Chronometer.
- Centrifuge.
- Pasteur pipette or vacuum system.
- Distilled water.
- Erythrocyte lysing solution.
- Wash buffer: phosphate buffered saline (PBS) + 0,09% (m/v) of NaN_3 + 0,5% (m/v) of Bovine Serum Albumin (BSA).

Preparation

Whole blood sample must be taken aseptically by means of a venipuncture [8, 9] in a sterilized tube for blood collection containing an appropriate anticoagulant (use of EDTA is recommended). 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. **The LC12 kit includes surface membrane (Sm) immunoglobulins (Ig) staining, therefore samples to be studied must be washed twice to remove the soluble serum proteins (steps 1a-1i). Be careful with volumes after discarding supernatants.**
    a. Pipette 50 µl of sample into a test 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.
    b. Add 4 mL of filtered PBS + 0,09% (m/v) of NaN_3 + 0,5% (m/v) of 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 4 mL PBS + 0,09% (m/v) of NaN_3 + 0,5% (m/v) 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.
2. Add 50 µl of the pre-mixed cocktail of 12 conjugated antibodies from a reconstituted vial.
3. Mix well.
4. Incubate for 30 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, leaving approximately 50 µl residual volume in each tube.
10. Wash by adding 4 ml of PBS + 0,09% (m/v) of NaN_3 + 0,5% (m/v) 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, leaving approximately 50 µl residual volume in each tube.
14. Resuspend the cell pellet in 200 µl of PBS + 0,5% (m/v) of BSA (without NaN_3).
15. Acquire directly on the flow cytometer at medium flow rate within the first hour after finishing the sample preparation. If the samples are not acquired immediately after preparation, they should be stored in the dark at 4-8 ºC for maximum 1 hour.

Important recommendations:

It is recommended following the Calibration EuroFlow Standard Operating Protocol for Cytometer Setup [6]. You will find a complete guide (Cytometer Setup SOP) on the web site www.EuroFlow.org, which includes recommendations for fixing instrument configuration, FSC and SSC setting, target voltage PMT setting, compensation setting and instrument performance monitoring.
An appropriated compensation setting is required for the appropriate analysis of this tube. Most fluorochromes emit also in surrounding inappropriate channels but this spillover can be mathematically corrected. Single stained tubes are used for compensation settings. For this purpose a liquid form sample of single reagents: CD45-OC515 (positive target population: lymphocytes), CD19-PE-Cyanine7 (positive target population: B-cells) and CD38-APC-C750 (positive target population: $CD38^{hi}$ lymphocytes population) are included in the kit. Use 5µl of each of these reagents in order to prepare the single stained tubes.

Flow cytometry analysis

Analysis of the LC12 files could become complicated with a manual definition of gates and regions, because different cell populations are present in the same fluorescence. Cytognos recommends the use of the **analysis software Infinicyt™**, which is capable to use pattern recognition and store analysis strategies to apply in batch to other samples using always the same criteria. You will find complete information about Infinicyt™ on the web site: www.infinicyt.com.

In order to perform the analysis using the Infinicyt software we recommend to follow these indications:

1. Select B cells (CD19+) as the first population to identify. LC12 kit includes CD19 PE-Cyanine7 alone in this channel, therefore a selection of B cells is clear to gate. Once identified, one can characterize the smIgλ-FITC/ smIgκ-PE ratio, but also the CD20, CD38, CD5 and CD10 expression of B cells.
2. Once B cells are classified, it is recommended to ¨hide¨ this population by deactivating its visibility in the profile and continue with the analysis of T cells.
3. Select T cells by their CD3+ expression and low FSC/SSC. Once identified, classify the T cell subsets using the other T cell markers included in the mixture of antibodies (CD4-Pacific Blue™ and CD8-FITC).
4. Once T and B cells are classified, it is recommended to ¨hide¨ these populations by deactivating their visibility in the profile and continue with the analysis of NK cells.
5. NK cell population can be then clearly identified based on its CD56 positive expression since smIgκ-PE positive cells are not show if B cells are not visible in the 2DDotPlot.
6. Plasma cells population could be identified based on its CD38 strong positive expression.

LIMITATIONS

- It is advisable to acquire stained samples on the cytometer as soon as possible to optimize the results. Non viable 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 leucocytes.
- 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 lymphocyte subsets. We recommend to follow the EuroFlow antibody panels [1] together with the EuroFlow instrument set-up, sample preparation and data analysis procedures [6].
- 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 leucopenic 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 these antigens and its relation to the expression of other relevant antigens to carry out an adequate analysis [1-5, 10, 11]

QUALITY CONTROL

- To obtain optimum results it is advisable to verify the precision of pipettes and that the cytometer is correctly calibrated.

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
	Keep out of sunlight
	Consult instructions for use
	For research use only
	Batch code
	Catalogue number
	Manufacter

PRODUCED BY

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