LST
Lymphoid Screening Tube
Panel

<table>
<thead>
<tr>
<th>Pacific Blue™</th>
<th>OCS15™</th>
<th>FITC</th>
<th>PE</th>
<th>PerCP-Cytochrome 5.5</th>
<th>PE-Cyanine7</th>
<th>APC</th>
<th>APC-C750</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD20+ CD4</td>
<td>CD45</td>
<td>CD8+ SmIgλ</td>
<td>CD56+ SmIgK</td>
<td>CD5</td>
<td>CD19+ TCRγδ</td>
<td>CD3</td>
<td>CD38</td>
</tr>
</tbody>
</table>

Ref: CYT-LST

For in vitro diagnostic use

LST VIALS CONTAIN LYOPHILIZED PRODUCTS. READ CAREFULLY THE FOLLOWING INSTRUCTIONS FOR RECONSTITUTION:

The lyophilized LST kit preserves the stability of the pre-mixed combination of antibodies. Reconstitute each lyophilized vial containing the pre-mixed combination with 300 µL of distilled water. Mix thoroughly each reconstituted vial in a “roller” mixer for at least 30 minutes at room temperature before use. Spin down each reconstituted vial before each use. Unused volume of the reconstituted vials is stable during one month from reconstitution date if stored in the dark at 2-8 ºC.

THE COMPENSATION TUBES CONTAIN LYOPHILIZED REAGENTS. READ CAREFULLY THE FOLLOWING INSTRUCTIONS FOR RECONSTITUTION:

To reconstitute the lyophilized compensation tubes, add directly to the tube the corresponding volume of sample intended for compensation: peripheral blood (PB) or beads. Incubate 30 minutes at room temperature in the dark, and then proceed with conventional protocol: EuroFlow™ standard operating protocol (SOP) for cell surface staining in the case of PB (www.euroflow.org) or manufacturer protocol for beads.

INTENDED USE
Lymphoid Screening Tube (LST) kit is a combination 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 panel 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 kit must be used by flow cytometry qualified personnel.

PRINCIPLES OF THE PROCEDURE
Flow cytometry is a technology that allows to simultaneously evaluate different characteristics of a single cell. Flow cytometers use hydrodynamic or acoustic focusing to individually present cells to one or more laser beams. As cells are intercepted by light, a set of detectors recovers two types of signals: those generated by dispersed light (FSC/SSC), which mainly reflect cell size and internal complexity, and those related to fluorochromes light emission when cells are labelled. Recovered signals are then amplified by a series of linear and logarithmic amplifiers, and converted into electrical signals to be plotted.

The fluorochrome-labeled antibodies bind specifically to the antigens they are directed against, allowing for the detection by flow cytometry of the different cell subsets. The erythrocyte population, which could hinder the detection of the target population, is lysed by using a red blood cell lysing solution.
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. LST kit recognizes by flow cytometry the antigens CD45, CD3, CD56, CD4, CD8, CD5, CD20, CD19, TCRγδ, CD38, kappa light chains and lambda light chains present in the different lymphocyte subsets and plasma cells and can therefore be used in the characterization studies for immunophenotyping of lymphocytes and plasma cells. These studies are widely applied in the characterization and follow-up of different hematological malignancies (25).

REAGENT COMPOSITION
LST kit contains sufficient volume for 25 tests distributed in lyophilized vials of 5 tests each and includes:

- 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 CD8/SmlgA-FITC antibody, clone: UCHT-4/Polyclonal, isotype: IgG2a/----.
  - Anti-human CD56/SmlgK-PE antibody, clone: C5.9/Polyclonal, isotype: IgG2b/----.
  - Anti-human CD5-PerCP-Cyanine 5.5 antibody, clone: L17F12, isotype: IgG2a.
  - Anti-human CD19/ TCRγδ-PE-Cyanine7 antibody clone: 19-1/ TCR-1, isotype IgG1/IgG1.

- 4 lyophilized tubes for compensation of 1 test each for CD5-PerCP-Cyanine 5.5, CD45-OC515™, CD19/TCRγδ-PE-Cyanine7 and CD38-APC-C750 conjugates.

All components contain 0.09% (m/v) sodium azide (NaNO₂). Reagents are not considered sterile.

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

WARNINGS AND RECOMMENDATIONS
1. For in vitro diagnostic use.
2. 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.
3. It contains 0.09% (m/v) 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.

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.
- P501 Dispose of contents in a container in accordance with local/regional/national/international regulation.

4. All patient specimens and materials which they come into contact are considered biohazards and should be handled as if capable of transmitting infection (6) and disposed according to the legal precautions established for this type of product. It is also recommended handling 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.

5. Use of reagent with dilutions, incubation times or temperatures different from those recommended may cause erroneous results. Any such changes must be validated by the user.

6. Any serious incident relating to the product must be reported to Cytognos S.L. as well as the competent professional authority of the Member State in which the user is established.

**PROCEDURE**

**Material required but not included**
- 3 laser-equipped flow cytometer (8-color or more such as Omnicyt™) and appropriate computer hardware and software.
- Test tubes suitable for acquiring samples in the flow cytometer. Usually tubes with a rounded bottom for 5 ml, 12x75 mm are used.
- Automatic pipette and tips
- 10 mL tubes
- Chronometer
- Pasteur pipette or vacuum system.
- Vortex Mixer
- Centrifuge
- Lysing solution containing a fixative agent
- Washing buffer: filtered solution of phosphate buffered saline (PBS) containing 0.09% (m/v) NaN₃, 0.2% (m/v) bovine serum albumin (BSA) and 2 mM ethylenediaminetetraacetic acid (EDTA).
- Acquisition buffer: filtered PBS solution containing 0.2% (m/v) BSA and 2 mM EDTA (NaN₃-free).

**Preparation**
Sample must be collected in commercially available anticoagulant-treated tube. Use of EDTA is highly recommended [7]. All steps indicated below should be performed at room temperature (RT).

**Recommended procedure:**

1. The LST tube includes surface membrane (Sm) immunoglobulins (lg) staining. Samples must be washed three times to remove the soluble serum proteins (steps 1a-1p) to avoid nonspecific staining.
   a. Pipette 300 µL of sample into a test tube.
   b. Add 6 mL of washing buffer.
   c. Mix well.
   d. Fill the tube up to 10 mL (by adding washing buffer).
   e. Centrifuge for 5 min at 540 g.
   f. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet.
   g. Add 6 mL of washing buffer to the cell pellet.
   h. Mix well.
   i. Fill the tube up to 10 mL (by adding washing buffer).
   j. Centrifuge for 5 min at 540 g.
   k. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet.
   l. Add 6 mL of washing buffer.
   m. Mix well.
   n. Fill the tube up to 10 mL (by adding washing buffer).
   o. Centrifuge for 5 min at 540 g.
   p. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet, leaving approximately 300 µL residual volume.

2. Add 50 µL of the pre-mixed cocktail of 12 conjugated antibodies from a reconstituted vial to 50µL of the cell pellet.
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. Add 2 mL of washing buffer 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 with acquisition buffer:
   - 320 µL using Omnicyt™
   - 200 µL using other flow cytometers
15. Acquire the cells immediately after staining or store at 4°C (for 1h maximum) until measured in the flow cytometer.
16. Acquire the sample at medium flow rate.
Important recommendations
In order to achieve optimal results, EuroFlow Standard Operating Protocol (SOP) for Cytometer Setup \(^{8}\) should be followed. You will find a complete guide on the web site www.euroflow.org, which includes recommendations for FSC, SSC and target voltage PMT settings, compensation setup and instrument performance monitoring.

Flow cytometry analysis
Analysis of the LST 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 Infinicyt\(^{TM}\) analysis software, 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\(^{TM}\) on the web site: www.infinicyt.com.

To analyze the results of LST tube we recommend follow these indications:
1. Select T cells (CD3+) as the first population to identify. LST kit includes CD3-APC alone in this fluorochrome, therefore a selection of T cells is clear to gate.
2. Gating T cell population, select and classify T cell subsets using the other T cell markers included in the mixture of antibodies (CD4-Pacific Blue\(^{TM}\), CD8-FITC, TCR\(\gamma\delta\), etc).
3. Once T cells are classified, it is recommended not show these cells on the screen and continue with the analysis of B cells.
4. B cell population can be then clearly identified based on their CD20 and CD19 positive expression since other markers in this fluorochrome are not show if T cells are not visible in the two dimensional DotPlot (2DDotPlot).
5. Gating B cell population, select and classify B cell subsets using the other B cell markers included in the mixture of antibodies (SmIg\(\kappa\)-FITC, SmIgk-PE).
6. Once T and B cells are classified, it is recommended not show these cells on the screen and continue with the analysis of NK cells.
7. NK cell population can be then clearly identified based on its CD56 positive expression since SmIgk-PE positive cells are not show if B cells are not visible in the 2D DotPlot.
8. Plasma cells population could be identified based on its CD38 positive expression.

PERFORMANCE CHARACTERISTICS
Specificity
- The CD3 antigen is expressed on the cell surface of mature thymocytes and T lymphocytes in peripheral blood.
- The CD4 antigen is expressed on a T-lymphocyte subpopulation in peripheral blood, most of thymocytes and some malignant cells of T-cell origin. Monocytes and macrophages show a weak expression of CD4. Normal B lymphocytes and granulocytes do not express surface CD4 antigen.
- The CD8 antigen is present on a T-lymphocyte subset in peripheral blood, 60% of thymocytes and a limited number of malignancies of T-cell origin. Normal B lymphocytes, monocytes and granulocytes do not express surface CD8 antigen.
- The CD19 antigen is expressed on the cell surface of normal and neoplastic B cells, and it is not expressed by T cells, monocytes and granulocytes.
- Anti-Kappa Light Chains react with free kappa light chains as well as intact immunoglobulin molecules bearing kappa light chains.
- Anti-Lambda Light Chains react with free lambda light chains as well as intact immunoglobulin molecules bearing lambda light chains.
- The CD56 antigen is expressed on all natural killer cells (activated and resting) in human peripheral blood and also in a CD3+ T cell subset.
- CD5 antigen is expressed on T lymphocytes but not on NK cells. Expression of CD5 antigen is determinant for the characterization of several hematological malignancies.
- TCR\(\gamma\delta\) (gamma-delta T-cell receptor) is expressed by peripheral blood T-cell subsets and thymocytes.
- CD38 antigen is expressed on plasma cells, activated T cells, monocytes, dendritic cells and macrophages. Expression of CD38 helps characterize several hematological malignancies.
- CD20 antigen is present on B lymphocytes and it is not present on plasma cells.
- CD45 antigen recognizes human leukocytes including lymphocytes, monocytes, granulocytes, and eosinophils. Erythrocytes, platelets and non-hematopoietic cells do not express CD45 antigen.
Expected values
Each laboratory should establish its own normal reference ranges for lymphocyte subset counting, since such values may be influenced by age, sex and race. The reference ranges for the different lymphocyte subsets shown in the following table are expressed as the percentage of lymphocyte populations. Data correspond to n = 69 whole blood samples from healthy donors acquired in a BD FACSCanto II cytometer and analyzed using Infinicyt™ software (Cytognos SL, Salamanca, Spain).

<table>
<thead>
<tr>
<th>Cell population</th>
<th>Reference population</th>
<th>Mean (%) ± SD (range)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>leucocytes</td>
<td>27.55 ± 7.66 (14.91 - 58.71)</td>
<td>27.8</td>
</tr>
<tr>
<td>B-cells</td>
<td>lymphocytes</td>
<td>10 ± 3.32 (0.96 - 18.3)</td>
<td>33.17</td>
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<tr>
<td>Kappa B-cells</td>
<td>B-cells</td>
<td>59.43 ± 5.25 (49.79 - 77.89)</td>
<td>8.83</td>
</tr>
<tr>
<td>Lambda B-cells</td>
<td>B-cells</td>
<td>40.57 ± 5.25 (22.11 - 50.21)</td>
<td>12.94</td>
</tr>
<tr>
<td>T-cells</td>
<td>lymphocytes</td>
<td>80.92 ± 6.46 (64.3 - 92.72)</td>
<td>7.98</td>
</tr>
<tr>
<td>CD4+ T-cells</td>
<td>T-cells</td>
<td>63.24 ± 11.87 (32.46 - 79.42)</td>
<td>18.77</td>
</tr>
<tr>
<td>CD8+ T-cells</td>
<td>T-cells</td>
<td>30.23 ± 9.51 (13.65 - 60.57)</td>
<td>31.46</td>
</tr>
<tr>
<td>TCRγδ+ T-cells</td>
<td>T-cells</td>
<td>1.28 ± 0.47 (0.83 - 1.72)</td>
<td>36.86</td>
</tr>
<tr>
<td>NK-cells</td>
<td>lymphocytes</td>
<td>9.08 ± 5.84 (1.02 - 23.56)</td>
<td>64.37</td>
</tr>
</tbody>
</table>

The following table shows the expected Mean Fluorescence Intensity (MFI) of the different antibodies included in this LST kit regarding target population. Data correspond to n = 69 whole blood samples from healthy donors acquired in a BD FACSCanto II cytometer and analyzed using Infinicyt™ software (Cytognos SL, Salamanca, Spain).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Dye</th>
<th>Cell population</th>
<th>Mean (MFI) ± SD (range)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAPPA</td>
<td>PE</td>
<td>CD20+/CD19+/CD3- B cells</td>
<td>35932.05 ±14492.92 (11453.54-62583.85)</td>
<td>40.33</td>
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<tr>
<td>LAMBDA</td>
<td>FITC</td>
<td>CD20+/CD19+/CD3- B cells</td>
<td>14508.00±2872.06 (8304.31-22974.82)</td>
<td>19.80</td>
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<tr>
<td>CD3</td>
<td>APC</td>
<td>CD3+/CD4+ T cells</td>
<td>41327.14±48539.73 (21726.62-64884.06)</td>
<td>20.66</td>
</tr>
<tr>
<td>CD4</td>
<td>Pacific Blue™</td>
<td>CD3+/CD4+ T cells</td>
<td>6308.25±702.35 (4839.15-7782.59)</td>
<td>11.13</td>
</tr>
<tr>
<td>CD5</td>
<td>PerCP-Cyanine5.5</td>
<td>CD3+/CD4+ T cells</td>
<td>17369.24±3751.54 (7340.00-27866.14)</td>
<td>21.60</td>
</tr>
<tr>
<td>CD8</td>
<td>FITC</td>
<td>CD3+/CD8++ T cells</td>
<td>16727.48±1862.44 (13265.94-21821.61)</td>
<td>11.13</td>
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<tr>
<td>TCRγδ</td>
<td>PE-Cyanine7</td>
<td>TCRγδ+/CD3++ T cells</td>
<td>3792.11±406.43 (3281.41-4268.73)</td>
<td>10.72</td>
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<tr>
<td>CD19</td>
<td>PE-Cyanine7</td>
<td>CD20+ B cells</td>
<td>17288.82±2050.09 (13570.84-21952.09)</td>
<td>11.86</td>
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<tr>
<td>CD20</td>
<td>Pacific Blue™</td>
<td>CD19+ B cells</td>
<td>18524.62±3411,86 (13008.73-27729.78)</td>
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<td>CD56</td>
<td>PE</td>
<td>CD56+/CD3-/CD19- NK cells</td>
<td>5712.40±1418.14 (3330.04-10184.72)</td>
<td>24.83</td>
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<tr>
<td>CD38</td>
<td>APC-C750</td>
<td>CD56+/CD3-/CD19- NK cells</td>
<td>2190.42±1435.50 (414.48-5901.26)</td>
<td>65.54</td>
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<tr>
<td>CD45</td>
<td>OC515™</td>
<td>CD45+/'SSC+™' lymphocytes</td>
<td>4502.99±1044.62 (2922.78-7027.39)</td>
<td>23.20</td>
</tr>
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</table>

Accuracy
Main lymphocyte subset percentages obtained with Cytognos LST screening tube were compared with results obtained with the reference combination proposed by EuroFlow consortium (1). The comparison of n=69 samples with both methods shows that LST is substantially equivalent. Data were analyzed with Infinicyt software and the following table indicates that the results are substantially equivalent in their reactivity on peripheral blood samples in terms of percentage of the different lymphoid subsets. Data were analyzed with Infinicyt™ software.
Twelve different whole blood samples from healthy donors stained with 2 different lots of LST screening tube were assessed. Each pair of data was analyzed to evaluate MFI differences of the different antibodies included in this LST kit.

Data were analyzed with Infinicyt™ software.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Fluorochrome</th>
<th>Cell population</th>
<th>Lot</th>
<th>Average MFI</th>
<th>% MFI differences</th>
<th>SD</th>
<th>CV (%)</th>
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<td>KAPPA</td>
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<td>APC-C750</td>
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</table>
LIMITATIONS

- It is advisable to acquire stained samples as soon as possible to optimize results. Non-viable cells may show unspecific staining. Prolonged exposure of samples to lytic reagents may cause white cell destruction and targeted population cell loss.
- When using whole blood lysing procedures some red blood cells may not lyse, for instance if there are nucleated red blood cells or if abnormal protein concentration and hemoglobinopathies are observed. This may cause misleading results since unlysed red blood cells are counted as leukocytes.
- Results obtained by flow cytometry may be erroneous if the cytometer lasers are misaligned or if gates are incorrectly set.
- Knowledge of antigen normal expression pattern and its relation to other relevant antigens is paramount to carry out an adequate analysis. (1-5, 10, 11)

QUALITY CONTROL

- Pipettes precision and cytometer calibration should be verified to obtain optimal results.
- In multicolor panels, fluorochromes emit in wavelengths that can show certain spectral overlap which must be corrected by electronic compensation. Optimal compensation levels can be established by analyzing cells from healthy individuals stained with mutually exclusive monoclonal antibodies conjugated with appropriate fluorochromes.
- This product has been manufactured in accordance with standards of production and quality system of the ISO 13485:2016 standard.

REFERENCES


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

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>🍀</td>
<td>Use by [YYY-MM]</td>
</tr>
<tr>
<td>📜</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>📞</td>
<td>Batch code</td>
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<tr>
<td>🌞</td>
<td>Keep out of sunlight</td>
</tr>
<tr>
<td>🔄</td>
<td>Storage temperature limitation</td>
</tr>
<tr>
<td>📋</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>🛡</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>🚨</td>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td>🔍</td>
<td>Contains sufficient for in vitro tests</td>
</tr>
<tr>
<td>⚠️</td>
<td>Health hazard/Hazardous to the ozone layer</td>
</tr>
</tbody>
</table>

### PRODUCED BY

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