SST
Small Sample Tube
Panel

EuroFlow

Pacific Blue™	OC515™	FITC	PE	PerCP-Cyanine 5.5	PE-Cyanine7	APC	APC-C750™
CD20	CD45	CD8+ SmIgλ	CD56+ SmIgK	CD4	CD19	CD3+ CD14	CD38

Ref: CYT-SST-R

RUO

For Research Use Only. Not for use in diagnostic procedures

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

The lyophilized SST 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**.
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
Small Sample Tube (SST, Small Sample Tube) is a kit with 11 conjugated antibodies designed for the detection of normal and aberrant lymphocyte populations of B, T and NK lineage by flow cytometry. This 8-color combination can be used for evaluation of "small" samples and samples with (very) low cell counts, such as fine needle aspirates (FNA), cerebrospinal fluid (CSF), vitreous humor, etc. with a clinical suspicion of primary lymphoma. SST is designed as part of the EuroFlow SST tube [(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
SST kit recognizes by flow cytometry the antigens CD20, CD45, CD8, CD56, CD4, CD19, CD3, CD14, 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. In small samples and samples with low cell counts, these studies are applied in the characterization and follow-up of primary lymphoma [2-10].

REAGENT COMPOSITION

SST 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 CD20-Pacific Blue™ antibody, clone: 2H7, isotype: 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 CD4-PerCP-Cyanine 5.5 antibody, clone: RPA-T4, isotype: IgG1.
  - Anti-human CD19-PE-Cyanine7 antibody clone: SA287, isotype IgG1.
  - Anti-human CD3/CD14-APC antibody, clone: UCHT-1/47-3D6, isotype: IgG1/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**	**CD45**	**CD8**	**SmIgλ**	**CD56**	**SmIgκ**	**CD4**	**CD19**	**CD3**	**CD14**	**CD38**
Clone	2H7	GA90	UCH-T4	Polyclonal	C5.9	Polyclonal	RPA-T4	SA287	UCHT-1	47-3D6	LD38
Isotype	IgG2b	IGg2a	IgG2a		IgG2b		IgG1	IgG1	IgG1	IgG1	IgG1
Reactivity	B cells	Leukocytes	Cytotoxic T cells	Lambda Ig light chain	NK cells	Kappa Ig light chain	Helper T cells	B cells	T cells	Monocytes	Plasma cells and others

- **4 lyophilized tubes for compensation** of 1 test each for the CD4-PerCP-Cyanine 5.5, CD45-OC515™, CD19-PE-Cyanine7 and CD38-APC-C750™ conjugates.

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

STORAGE CONDITIONS

The reagent is stable until the expiration date shown on the label, when stored at 2-8 ºC. 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 research use only. Not for use in diagnostic procedures.
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 [11] 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, 12x 75 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) NaN_3, 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 (NaN_3-free).

Preparation

Small sample must be taken aseptically by means of lumbar puncture [12] in case of CSF sample, vitrectomy or fine needle aspirate in a sterilized tube. Store samples at 4º-8ºC and processed within 1 hour after their extraction; otherwise they should be stabilized to avoid deterioration of cells.

Recommended procedure:

1. **The SST kit includes surface membrane (Sm) immunoglobulins (Ig) 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 tube completely at medium flow rate.

Important recommendations

In order to achieve optimal results, EuroFlow Standard Operating Protocol (SOP) for Cytometer Setup [13] 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 SST 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.

To analyze the results of SST tube we recommend follow these indications:

1. Exclude debris and non-leukocyte events from analysis by gating on forward light scatter (FSC), side light scatter (SSC) and preferably CD45. Plasma cells may have CD45 negative/dim expression, therefore in that case, include also CD45low positive cells in the leukocyte gate
2. Select cells of interest using a lineage-specific marker and side scatter (SSC) and preferably a dual-anchor gating strategy using CD45 versus SSC. Analyze un-gated data as well.
3. Use normal lymphocytes, monocytes, and neutrophils within the sample as an internal control for negative or positive antigen expression.
    a) Analyze B cell data for abnormal patterns of antigen expression and/or light scatter characteristics (large-cell lymphoma) using CD19 and CD20 (in some occasions CD19 is weak or negative so use of CD20 is advisable). Analyze light-chain expressions within the abnormal population in combination with CD19 and CD20. Populations are classified as monoclonal (or showing clonal excess) when a surface immunoglobulin (smIg) Kappa/Lambda ratio below 0.25 or above 4 is observed, provided that enough (n > 100) smIg+ B cells have been measured. Some cases, i.e., diffuse large B cell lymphoma, may be light-chain negative.
    b) Analyze T cell data looking for abnormal patterns of antigen expression as well as light-scatter characteristics.
4. Sensitivity and minimum number of events to define leukocyte infiltration in the sample vary depending on the number of events simultaneously assessed.

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. [1-10]

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.
- To evaluate the non-specific binding of the reagent, an appropriated isotype control tube can be prepared.
- This product has been manufactured in accordance with standards of production and quality system of the ISO 9001:2015 standard.

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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)
REF	Catalogue number
LOT	Batch code
	Keep out of sunlight
	Storage temperature limitation
	Consult instructions for use
	Manufacturer
RUO	For research use only
Σ	Contains sufficient for <n> tests
	Health hazard/Hazardous to the ozone layer

PRODUCED BY

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