SST
Small Sample Tube

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

CE

For in vitro diagnostic use

SST VIALS ARE A LYOPHILIZED PRODUCT. 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**. Unused volume of reconstituted vial is stable during one month from date of reconstitution if it is stored at 2º-8º C.

INTENDED USE

Small Sample Tube (SST) 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-colour panel 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 reagent must be used by flow cytometry qualified personal.

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 measurement of antigen expression.

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)].

The use of 8 color panels in flow cytometry involves the use of new fluorochromes here described:

- Orange Cytognos 515 is a fluorochrome excited with the violet laser (405nm). Orange Cytognos 515 emits at 515nm. This fluorochromes provide maximum resolution and narrow emission peaks, which results in little spectral overlap and minimal compensation requirements.
- APC-C750 is a tandem dye with a maximum emission peak at 779 nm, which grants bright signal, low unspecific noise and high photostability. When excited by light from a red laser, the APC fluorochrome can transfer energy to C750 molecule, which then emits at a longer wavelength. It is recommended to use a 780/60 nm bandpass filter along with a red sensitive detector to use in conjunction antibodies conjugated with APC and APC-C750.

PRINCIPLES OF THE PROCEDURE

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 present in the reagents bind specifically to the antigens they are directed against, allowing the detection by flow cytometry of the different lymphoid subsets.

The 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.

It is recommended follow the Calibration EuroFlow Standard Operating Protocol for Cytometer Setup [11]. You will find a complete guide (Cytometer Setup SOP) on the web site www.euroflow.org, which includes recommendations for fix instrument configuration, FSC and SSC setting, target channel PMT setting, compensation setting and instrument performance monitoring.

REAGENT COMPOSITION

SST kit contains sufficient volume for 25 tests distributed in lyophilized vials of 5 tests each. SST 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 CD20-Pacific Blue™ antibody, clone: 2H7, isotype: IgG2b
- Anti-human CD45-OC515 antibody, clone: GA90, isotype: IgG2a.
- Anti-human CD8/IgmA-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: SK3, isotype: IgG1.
- Anti-human CD19-PE-Cyanine7 antibody clone: 19-1, isotype IgG1.
- Anti-human CD3/CD14-APC antibody, clone: 33-2A3/47-3D6, 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	SK3	19-1	33-2A3	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

Additionally, CD45-OC515, CD19-PE-Cyanine7 and CD38-APC-C750 compensation vials of 5 tests. These compensations vials are in liquid format ready to use. Compensation requirements for OC515, PE-Cyanine7 and APC-C750 are similar to Pacific Orange™, PECy7 and APC-H7 respectively.

All components contain <0,1% (m/v) sodium azide (NaN_3). Reagents are not considered sterile.

STORAGE CONDITIONS

CYT-SST is stable until the expiration date shown on the label, when are stored at 2-8º C. The expiration date applies to the lyophilized product. Vials with the pre-mixed cocktail of 11 conjugated antibodies are stable one month from date of reconstitution.
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

Reconstitute each lyophilized vial containing the pre-mixed cocktail of 11 conjugated antibodies **with 300 µl of distilled water**. Unused volume of reconstituted vial is stable during one month from date of reconstitution if it is stored at 2º-8º C.

WARNINGS AND RECOMMENDATIONS

1. For *in vitro* diagnostic use.
2. This product is supplied ready to use, any modification by dilution or addition of other components, it will be invalidated for *in vitro* diagnostic use.
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 or discoloration indicates instability or deterioration. In such cases, the reagents should not be used.
5. It contains <0,1% (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.
   - 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 [12], 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 S.L. as well as the competent professional authority of the Member State in which the user is established.

PROCEDURE

Material included

CYT-SST is sufficient for 25 determinations. It includes the following reagents:

- **5 Lyophilized vials** with a pre-mixed cocktail of 11 conjugated antibodies.
- Additionally, CD45-OC515, CD19-Cyanine7 and CD38-APC-C750 **compensation vials** of 5 tests. These compensations vials are in liquid format 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

- Flow cytometer equipped with 405 nm violet laser, 488 nm ion argon laser, 633 nm red laser, 780/60 nm bandpass filter, and appropriate computer hardware and software associated.
- Test tubes suitable for obtaining samples in the flow cytometer used. Usually tubes with a rounded bottom for 6 ml, 12x75 mm are used.
- Automatic pipette (100 µl) and tips.
- Micropipette with tips.
- Vortex Mixer.
- Chronometer.
- Centrifuge.
- Pasteur pipette or vacuum system.
- Distilled water.
- Isotypic control reagent.
- Erythrocyte lysing solution.
- Wash buffer as phosphate buffered saline (PBS) + 0.09% of NaN_3 (m/v) + 0.5% (m/v) of Bovine Serum Albumin (BSA).

Preparation

Small sample must be taken aseptically by means of lumbar puncture [10] 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.

1. **The SST panel includes surface membrane (Sm) immunoglobulins (Ig) staining and immunoglobulins (Ig) staining, therefore samples must be washed twice to remove the soluble serum proteins (steps 1a-1j). Be careful with volumes after discarding supernatants.**
    a. Spin down total volume of the small sample (i.e. CSF, vitreous aspirates) during 5 min at 540 xg. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet.
    b. Add 2-5 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 xg.
    e. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet.
    f. Add 2-5 ml of filtered PBS + 0.09% (m/v) of NaN_3 + 0.5% (m/v) of BSA.
    g. Mix well.
    h. Centrifuge for 5 min at 540 xg.
    i. Discard the supernatant using a Pasteur pipette or vacuum system without disturbing the cell pellet.
    j. Resuspend cell pellet in 150 µl of PBS + 0.09% (m/v) of NaN_3 + 0.5% (m/v) of BSA.

2. Pipette 50 µl of this sample in a new tube and add 50 µl of the pre-mixed cocktail of 11 conjugated antibodies from a reconstituted vial.
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 540 xg.
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 2 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 540 xg.
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% of BSA (without NaN_3).
15. Acquire the cells after staining or (if not immediately acquired) store at 4ºC for maximally 1 h until measured in the flow cytometer.

This is the detailed EuroFlow Standard Operating Procedure for sample preparation and staining. Other staining protocols must be validated for the use of this reagent.

Important recommendations:

It is recommended following the Calibration EuroFlow Standard Operating Protocol for Cytometer Setup [11]. 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 channel PMT setting, compensation setting and instrument performance monitoring.

An appropriated compensation setting is required for the acquisition 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 sample of CD45-OC515 (positive target population: lymphocytes), other of CD19-PE-Cyanine7 (positive target population: B-cells) and another of 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 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 a 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) smlg+ 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

- Small samples should be stored at 18-22ºC and processed within 24 hour after their extraction; otherwise they should be stabilized to avoid deterioration of cells.
- 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 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 lymphocyte subsets and plasma cells using its own test conditions. We recommend follow the EuroFlow antibody panels [1] together with the EuroFlow instrument set-up, sample preparation and data analysis procedures [11].
- Relative concentration of cell samples isolated by density gradient could be different from the origin samples. Those differences could be relatively insignificant in samples from patients with normal cell count. In leukopenia patients, selective loss of subpopulations could alter 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.
- It is recommended follow the Calibration EuroFlow Standard Operating Protocol for Cytometer Setup. You will find a complete guide (Cytometer Setup SOP) on the web site www.euroflow.org, which includes recommendations for fix instrument configuration, FSC and SSC setting, target channel PMT setting, compensation setting and instrument performance monitoring.
- 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 13485:2012 standard.

PERFORMANCE CHARACTERISTICS

Specificity

The selection of antibodies for the SST aimed at screening for lymphoma in "small" samples and samples with (very) low cell counts obtained from suspected cases. The SST panel enables optimal detection and identification of all possible cell types in pauci-cellular materials, a series of antibodies had to be included in a single tube. Thus, the markers that were selected included:

-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.
-CD14 antigen also known as LPS receptor is expressed on monocytes/macrophage cells and with low intensity on granulocytes. CD14 is also expressed on some dendritic cell populations.
-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 and monocytes 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 = 22 whole blood samples from healthy donors acquired in a BD FACSCanto II cytometer and analyzed using Infinicyt™ software (Cytognos SL, Salamanca, Spain).

Cell population	Reference population	Mean (%) ± SD (range)	CV (%)
Lymphocytes	Leucocytes	21.59 ± 7.95 (11.02 – 36.91)	36.85
B-cells	Lymphocytes	14.41 ± 9.44 (5.14- 50.14)	65.5
Kappa B cells	B-cells	57.41 ± 6.27 (45.91-74.85)	10.92
Lambda B-cells	B-cells	41.53 ± 6.56 (22.44-54.09)	15.8
T-cells	Lymphocytes	85.59 ± 9.44 (49.26-94.86)	11.03
CD4+ T-cells	T-cells	63.26 ± 7.95 (44.43-74.35)	12.56
CD8++ T-cells	T-cells	29.82 ± 7.14 (20.45-49.85)	23.94
CD14+ cells	Monocytes	5.2 ± 1.22 (3.09-8.55)	25.3
NK-cells	Lymphocytes	1.69 ± 0.97 (0.42-4.05)	57.26

The following table shows the expected Mean Fluorescence Intensity (MFI) of the different antibodies included in this SST kit regarding target population. Data correspond to n = 22 whole blood samples from healthy donors acquired in a BD FACSCanto II cytometer and analyzed using Infinicyt™ software (Cytognos SL, Salamanca, Spain).

Antibody	Fluorochrome	Cell population	Average MFI ± SD (Range)	CV (%)
KAPPA	PE	CD20+/CD19+/CD3- B cells	32158.24 ± 20337.86 (4174.27 – 65219.67)	63.24
LAMBDA	FITC	CD20+/CD19+/CD3- B cells	15187.42 ± 4086 (4907.60 – 22860.07)	26.91
CD3	APC	CD3+/CD4+ T cells	36915.04 ± 7487.05 (28371.23 – 54788.87)	20.28
CD4	PerCP-Cyanine5.5	CD3+/CD4+ T cells	23051.84 ± 4364.30 (11068.87 – 28177.89)	18.93
CD14	APC	CD14+/CD4+/CD45+/SSC^{Med}	75081.77 ± 23483.19 (38323.20 – 126653.51)	31.28
CD8	FITC	CD3+/CD8++ T cells	16875.74 ± 1742.21 (14314.21 – 20683.68)	10.32
CD19	PE-Cyanine7	CD19+ B cells	16320.19 ± 2097.79 (11597.63 – 20161.40)	12.85
CD20	Pacific Blue™	CD20+ B cells	20758.79 ± 4555.98 (7828.16 – 27825.71)	21.95
CD56	PE	CD56+/CD3-/CD19- NK cells	4317.94 ± 1518.42 (2015.78 – 7810.03)	35.17
CD38	APC-C750	CD56+/CD3-/CD19- NK cells	2422.51 ± 1757.86 (455.96 – 2168.94)	72.56
CD45	OC515	CD45+/SSC^{Low} lymphocytes	5535.25 ± 777.56 (4340.81 -7276.63)	14.05

Accuracy

Main lymphocyte subset percentages obtained with Cytognos SST screening tube were compared with results obtained with the reference combination proposed by EuroFlow consortium [(1)]. The comparison of n=22 samples with both methods shows that SST 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.

Cell population	Reference population	Control Method Mean (%) ± SD (range)	Cytognos SST Mean (%) ± SD (range)	Mean differences (%)	CV Control (%)	CV Cytognos (%)	p-value*
Lymphocytes	Leucocytes	23.87 ± 8.73 (10.71-36.08)	21.59 ± 7.95 (11.02 -36.91)	2.28	36.58	36.85	0.24
B-cells	Lymphocytes	16.28 ± 12.42 (8.33-53.21)	14.41 ± 9.44 (5.14-50.74)	1.87	76.34	65.5	0.35
Kappa B-cells	B-cells	56.48 ± 8.39 (46.76-76.29)	57.41 ± 6.27 (45.91-74.85)	0.93	14.85	10.92	0.56
Lambda B-cells	B-cells	40.82 ± 8.76 (17.78-52.75)	41.53 ± 6.56 (22.44-54.09)	0.71	21.46	15.8	0.65
T-cells	Lymphocytes	83.72 ± 6.56 (46.79-91.67)	85.59 ± 9.44 (49.26-94.86)	1.87	15.80	11.03	0.35
CD4+ T-cells	T-cells	65.38 ± 6.78 (53.51-73.55)	63.26 ± 7.95 (44.43-74.35)	2.12	10.37	12.56	0.29
CD8++ T-cells	T-cells	28.01 ± 5.75 (19.78-39.66)	29.82 ± 7.14 (20.45-49.85)	1.81	20.52	23.94	0.29
CD14+ cells	Monocytes	4.81 ± 7.14 (3.29-6.32)	5.2 ± 1.22 (3.09-8.55)	0.39	23.94	25.3	0.27
NK-cells	Lymphocytes	2.07 ± 1.15 (0.49-4.29)	1.69 ± 0.97 (0.42-4.05)	0.38	55.47	57.26	0.17

*No significant differences between groups ($p \geq 0.05$)

Repeatability

Ten different whole blood samples from healthy donors stained with 2 different lots of SST screening tube were assessed. Each pair of data was analyzed to evaluate MFI differences of the different antibodies included in this SST kit. Data were analyzed with Infinicyt™ software. The results of these analysis are shown in the following chart:

Antibody	Fluorochrome	Cell population	Lot	Average MFI	% MFI differences	SD	CV (%)	p-value
KAPPA	PE	CD20+/CD19+/CD3- B cells	**1**	26462,27	23.14	14894.45	56.29	0.05
			2	34430,79		26206.24	76.11	
LAMBDA	FITC	CD20+/CD19+/CD3- B cells	**1**	18692,04	25.39	2673.70	14.30	0.38
			2	14907,58		4698.35	31.52	
CD3	APC	CD3+/CD4+ T cells	**1**	36418,86	9.36	7606.15	20.89	0.18
			2	40177,98		6812.67	16.96	
CD19	PE-Cyanine7	CD19+ T cells	**1**	17542,98	5.86	1475.95	8.41	0.97
			2	16571,74		2762.01	16.67	
CD14	APC	CD3+/CD4+ T cells	**1**	71359,28	12.60	21380.38	29.96	0.64
			2	81642,81		31817.81	38.97	
CD56	PE	CD56+/CD3-/CD19- NK Cells	**1**	3095,14	37.65	942.55	30.45	0.01
			2	4963,87		774.35	15.60	
CD8	FITC	CD3+/CD8++ T cells	**1**	17747,48	13.55	1260.56	7.10	0.04
			2	15629,03		1066.46	6.82	
CD45	OC515	CD45+/SSC^{Low} lymphocytes	**1**	4861,57	10.48	581.52	11.96	0.57
			2	5430,58		369.15	6.80	
CD20	Pacific Blue™	CD20+ B cells	**1**	21728,99	1.41	4319.60	19.88	0.88
			2	21427,89		4313.77	20.13	
CD4	PerCP-Cyanine5.5	CD3+/CD4+ T cells	**1**	22018,12	6.73	5531.10	25.12	0.25
			2	23607,98		983.29	4.17	
CD38	APC-C750	CD56+/CD3-/CD19-CD20/CD45hi/SSCLow NK cells	**1**	3095,14	107.3	942.55	30.45	0.002
			2	1492,67		474.10	31.76	

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EXPLANATION OF SYMBOLS

	Use by (YYYY-MM)
	Storage temperature limitation
	Keep out of sunlight
	Consult instructions for use
IVD	*In vitro* diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Manufacter

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