CD45-FITC CD4- RD1 CD8- ECD CD3-PC5 CD56+CD16)-RD1 CD19-ECD

**Specificity**
- CD45
- CD4
- CD8
- CD3
- CD56, CD16
- CD19

**Clone**
- B3821F4A
- SFC11274D11
- SFC12171yD03
- UCHT1
- N901NKH-1, 3G8
- J3-119

**Hybridoma**
- NS-1 x BALB/c
- NS-1 x BALB/c
- NS-1 x BALB/c
- NS-1 x BALB/c
- NS-1 x BALB/c
- NS-1 x BALB/c

**Immunogen**
- Transfectant containing cDNA of human CD45
- Human peripheral T lymphocytes
- Human thymocytes
- Human thymocytes and peripheral blood lymphocytes from a person with Sezary cell leukemia
- Human chronic myeloid leukemia cells, Human neutrophils
- SKLY 18 lymphoma cells

**Ig Chain**
- IgG2b
- IgG1
- IgG1
- IgG1
- IgG1
- IgG1

**Species**
- Mouse
- Mouse
- Mouse
- Mouse
- Mouse
- Mouse

**Source**
- Ascites fluid
- Conditioned media
- Conditioned media
- Ascites fluid
- Conditioned media
- Conditioned media

**Purification**
- Affinity chromatography
- Affinity chromatography
- Affinity chromatography
- Affinity chromatography (both)
- Affinity chromatography (both)

**Fluorescence**
- Excites at 486-580 nm
- Emits at 504-541 nm
- Excites at 486-580 nm
- Emits at 588-590 nm
- Excites at 486-580 nm
- Emits at 560-680 nm
- Excites at 486-580 nm
- Emits at 610-635 nm
- Excites at 486-580 nm
- Emits at 610-635 nm

**Conjugation**
- FITC (Fluorescein isothiocyanate)
- RD1 (Phycoerythrin)
- ECD (Phycoerythrin - Texas Red-X)
- PC5 (Phycoerythrin-Cy5)
- RD1 (Phycoerythrin - both)
- ECD (Phycoerythrin - Texas Red-X)

**Molar Ratio**
- FITC/Protein: 3-10
- RD1/Protein: 0.5-1.5
- ECD/Protein: 0.5-1.5
- PC5/Protein: 0.5-1.5
- RD1/Protein: 0.5-1.5
- ECD/Protein: 0.5-1.5

**MONOCLONAL ANTIBODY**

For In Vitro Diagnostic Use

Rx Only in the U.S.A.

**INTENDED USE**

AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel monoclonal antibody reagents are for use on the AQUIOS CL Flow Cytometer with peripheral whole blood for immunophenotyping. These reagents are indicated for use in the immunologic assessment of patients having, or suspected of having, immune deficiency. These reagents provide identification and enumeration of:

- **AQUIOS Tetra-1 Panel Monoclonal Antibody Reagents**
  - Total CD3+, CD3+CD4+, CD3+CD8+, CD3+CD4+/CD3+CD8+ (ratio only) lymphocyte percentages and absolute counts.
  - CD45+ absolute count
  - CD45+ Low SS (lymphocytes) percentage and absolute count

- **AQUIOS Tetra-2+ Panel Monoclonal Antibody Reagents**
  - Total CD3+, CD3-CD19+, CD3-CD56+ and/or CD16+ lymphocyte percentages and absolute counts.
  - CD45+ absolute count
  - CD45+ Low SS (lymphocytes) percentage and absolute count

Refer to the AQUIOS Tetra System Guide (PN B26364) for instructions on how to use these reagents in the system and their respective Performance Characteristics.

**SUMMARY AND EXPLANATION**

The leukocyte common antigen (CD45) is a transmembrane-type protein expressed at high levels on nucleated hematopoietic cells with the exclusion of megakaryocyte/platelet and erythroid series. CD45-assisted Leukocyte gating along with CD4 allow readily enumeration of absolute count and percentages of CD4 T cells. 1-3,44 The expression of CD45 density is useful for discriminating between normal and malignant leucocytes cells. The density of expression of CD45 is weak in some malignant cells (i.e. acute myeloid leukemias) thus, enabling malignant cells to be distinguished from normal ones.

The lymphocyte population of human peripheral blood is composed of three cell types: T (thymus-derived), B (bone marrow-derived), and NK (Natural Killer) cells. 1 These cell types are morphologically distinguishable by microscopy but can be identified by characteristic antigenic differences in their cell membranes.

T, B, and NK lymphocytes play central roles in immune system function. Different subtypes of T lymphocytes may recognize specific antigens, execute effector functions and/or control both the type and intensity of cellular and/or humoral immune responses. Upon activation by antigens or macrophages via T lymphocytes, specific B lymphocytes differentiate into plasma cells which produce and secrete specific immunoglobulins (Ig). NK lymphocytes have been identified as a discrete population of cytolytic effectors which may recognize specific antigens, execute effector functions and/or control both the type and intensity of cellular and/or humoral immune responses. Upon activation by antigens or macrophages via T lymphocytes, specific B lymphocytes differentiate into plasma cells which produce and secrete specific immunoglobulins (Ig).

The CD45+ cells are identified as white blood cells distinguished from normal ones.

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The CD45+ cells are identified as white blood cells distinguished from normal ones.

Identification of abnormal levels of CD4+ immunodeficiency, and corresponding CD4+/CD8+ ratios, might also aid in the diagnosis and/or prognosis of immunodeficiency disease. For example infection with human immunodeficiency virus (HIV), the etiologic agent of acquired immunodeficiency syndrome (AIDS), results in profound immunosuppression due predominantly to a selective depletion of the CD4+ lymphocytes that express the receptor for the virus. 17,34 Progressive clinical and immunologic deterioration generally correlates with a decreasing CD4+ lymphocyte count. 17

CD3+(CD56+CD16)+ Lymphocytes

NK lymphocyte populations have been functionally defined as a lymphocyte population capable of mediating non-MHC restricted cytotoxicity against targets such as certain tumor and virus-infected cells. 25

CD4/CD8 Ratio

Disease-related changes in CD4+ and CD8+ lymphocyte levels might alter CD4/CD8 inducer suppressor/cytotoxic cell ratios. As a result, CD4/CD8 ratios might be useful as diagnostic and/or prognostic indicators of immune competence.

CD4/CD8 ratios in conjunction with CD4+ lymphocyte cell numbers have been the most widely used laboratory parameters for evaluation of AIDS-related complex and AIDS. 17,26 CD4/CD8 ratios approach zero in advanced AIDS patients with no detectable levels of CD4+ lymphocytes. 17 In such cases, CD8+ lymphocyte levels might be normal, increased or decreased.

Decreased CD4+ and CD8+ lymphocyte percentages without significant changes in CD4/CD8 ratios have been observed in patients with stable renal allograft function after transplantation. 5 In addition, low CD4/CD8 ratios and decreased percentages of CD4+ lymphocytes have been documented in patients during phenotypic reconstitution following purged autologous bone marrow transplantation. 21,32

CD45+ Cells

The CD45+ cells are identified as white blood cells (leukocytes) since CD45 antigen is expressed on every type of hematopoietic cell except mature erythrocytes and their immediate progenitors 18,19,20 and is not identified on cells of non-hematopoietic origin. CD45 in combination with Side Scatter may be used to...
define leukocytes and to differentiate discrete cell populations for immunophenotyping.

**CD45+ Low SS (Lymphocytes)**
The CD45+ Low SS cells are identified as the lymphocyte population. The lymphocyte population can be further differentiated into discrete cell populations for immunophenotyping.

**Lymphocyte Immunophenotyping Panel**
AQUIOS Tetra-1 Panel provides the ability to enumerate an individual’s major lymphocyte subsets: T, B and NK. The reagent can function as a quality control check (LymphoSum) to estimate CD45SS Lymphocyte gate recovery and account for all lymphocyte subsets. Total lymphocyte percentage should be determined using the following formula:

\[
\text{Total Lymphocyte Percentage (\%)} = \%\text{CD3+ (T)} + \%\text{CD19 (B)} + \%\text{CD3- (CD56+CD16+) (NK)}
\]

Used as a panel, AQUIOS Tetra-1 Panel also functions as a quality control check for a specimen in terms of analysis of lymphocyte subpopulations. (Refer to the monoclonal antibody reagents provides automated analysis of lymphocyte subpopulations. (Refer to the AQUIOS Tetra System Guide PN B26364 for the minimum volume of blood required for analysis.

**PRINCIPLES OF TEST**
This test depends on the ability of a monoclonal antibody to bind to the surface of cells expressing discrete antigens. Specific cell staining is accomplished by incubating whole blood with the monoclonal antibody reagent. The AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel monoclonal antibody reagents are each a combination of four or five murine monoclonal antibodies, each conjugated to a specific fluorochrome and specific for different cell surface antigens.

Red blood cells are lysed with the AQUIOS Lysing Reagent Kit. The remaining white blood cells are analyzed by flow cytometry using lymphocyte gates. In the first histogram for either reagent, the lymphocyte gate is identified as having bright CD45+FITC fluorescence and low Side Scatter (SS).

For AQUIOS Tetra-1 Panel CD45-FITC/CD4-RED1/CD8-ECDF/CD3-PCS the following histograms/plots are used to determine the percentage of positively stained cells: CD3+ (positive PC5 fluorescence only), CD3+CD4+ (positive PC5/RED1 fluorescence), CD3+CD8+ (positive PC5/ECDF fluorescence).

For AQUIOS Tetra-2+ Panel CD45-FITC/CD56+CD16-RD1/CD19-ECDF/CD3-PCS, the following histograms/plots are used to determine the percentage of positively stained cells: CD3+ (positive PC5 fluorescence only), CD3-CD56+ and/or CD16+ (positive RD1 fluorescence only), CD3-CD19+ (positive ECDF fluorescence only).

The AQUIOS System Software with Tetra-1 and Tetra-2+ tests in conjunction with AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel monoclonal antibody reagents provides automated analysis of lymphocyte subpopulations. (Refer to the AQUIOS Tetra System Guide PN B26364 for more details.)

**REAGENTS**
See table on page 1.

**REAGENT CONTENTS**
The antibody concentration is 1.445/0.1820/365/0.365 μg/test for CD45-FITC/CD4-RED1/CD8-ECDF/CD3-PCS and 1.445/0.0220/0.0910/0.2470/0.365 μg/test for CD45-FITC/CD56-RED1/CD16-RED1/CD19-ECDF/CD3-PCS.

The concentration of nonantibody reagents is 0.2% BSA, 0.01 M potassium phosphate, 0.15 M NaCl, 0.1% NaN3 and stabilizers.

**STATEMENT OF WARNINGS**
Iodoacetamide <0.1%

May produce an allergic reaction.

1. These reagents contain 0.1% sodium azide. Sodium azide under acidic conditions yields hydrazodic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.

2. Specimens, samples, and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.

3. Do not remove the supplied Cap with Septa from the packaging until ready to use.

4. Ensure that the shipping cap has been replaced with the Cap with Septa immediately prior to loading on the system. Otherwise, damage will occur to flow cytometer system.

5. Never pipette by mouth and avoid contact of samples with skin and mucous membranes.

6. Do not use reagent beyond the expiration date on the vial label.

7. Minimize exposure of reagent to light during storage or incubation.

8. Avoid microbial contamination of reagent or erroneous results may occur.

9. Use Good Laboratory Practices (GLP) when handling reagent.

10. Review all data plots and histograms before reporting results. Refer to the AQUIOS Tetra System Guide (PN B26364) for the Flow Cytometry Gating Strategy and Data Plot Examples.

11. Harmful if swallowed.

12. After contact with skin wash immediately with plenty of water.

13. Do not operate the system with an uncapped vial of antibody reagent.

14. Once the cap with septa has been pierced the vial has the potential to leak if the bottle is not upright.

**STORAGE CONDITIONS AND STABILITY**
Unopened reagent is stable to the expiration date on the vial label when stored at 2-8°C. Opened vials are stable for 90 days when stored at 2-8°C. Return reagent to 2-8°C immediately after use. Do not freeze. Minimize exposure to light.

Monoclonal antibody reagent vials may be left at room temperature on board the AQUIOS System for up to a maximum of 80 total cumulative hours. If the facility operates the System for periods of longer than 8 continuous hours, it is suggested that the carousel be further differentiated into discrete cell populations for immunophenotyping.

**PROCEDURE FOR IMMUNOFLOURESCENCE CELL SURFACE STAINING WITH TETRA-1 PANEL AND TETRA-2+ PANEL**

**MATERIALS SUPPLIED**
AQUIOS Tetra-1 Panel
B23533 - 50 tests (0.9 mL)
AQUIOS Tetra-2+ Panel
B23534 - 50 tests (0.9 mL)
B30104 – Cap with Septa (1)

**MATERIALS REQUIRED BUT NOT SUPPLIED**
AQUIOS Lysing Reagent Kit, PN B23538 –100 tests
AQUIOS IMMUNO-TROL Cells, PN B23535
AQUIOS IMMUNO-TROL Low Cells, PN B25700

**PROCEDURE**
1. Bring the antibodies to 18-26°C.

2. If the vial has not been previously used on the system, remove the shipping cap and replace with the Cap with Septa provided in the packaging. Replacement of the shipping cap should only occur immediately prior to loading on the system to allow the system to monitor and accurately reflect the open vial stability claim.

3. LOAD the sample on the AQUIOS system. Select “Patient” in the “Test Request”.

   ■ For autoloader, insert tube(s) into the instrument cassette and place the cassette on the system.

   ■ For single tube loader, mix the sample immediately before placing the tube in the tube sampling area. Scan the tube and place on the system.

4. GO is automatically initiated when sample(s) are placed on the system.

   ■ For autoloader, the system runs the sample(s) when the cassette is loaded.
For single-tube loader, the system runs the sample(s) when the door is closed.
5. Allow the system to prepare the sample(s) and to analyze.

Refer to the AQUIOS Tetra System Guide PN B26364 for detailed instructions.

QUALITY CONTROL PROCEDURE
Daily Quality Control is a critical component of ensuring the system’s performance for the Tetra application. Refer to the AQUIOS Tetra System Guide PN B26364.

LIMITATIONS
1. The reagents are for use only on AQUIOS Flow Cytometers.
2. Sample staining must be prepared with AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel reagents and AQUIOS Lysing Reagent System within 24 hours of collection.
3. The CD45+ absolute count and CD45+ Low SS percentage and absolute counts should only be used for immunophenotyping flow cytometric analysis.
4. Retain specimens in blood collection tubes at room temperature prior to staining and analyzing.
5. Do not refrigerate specimens. Refrigerated specimens may give aberrant results.
6. The recommended cell viability for venous blood specimens is >90%.
7. AQUIOS Tetra-1 Panel and AQUIOS Tetra-2+ Panel and AQUIOS monoannelceal antibody reagents are designed for use with whole blood samples. They may also be used with AQUIOS IMMUNO-TROL Cells, and/or AQUIOS IMMUNO-TROL Low Cells. The reagents are not recommended for use with fresh or frozen mononuclear cell preparations.
8. Do not dilute, aliquot, or freeze the reagents. The product should be used according to labeled instructions.
9. In patients treated with anti-human monoclonal antibody therapies, detection of the specific targeted antigens may be diminished or absent due to partial or complete blocking by the treatment antibody.
10. Abnormal states of health are not always represented by abnormal percentages of certain leucocyte populations. An individual in an abnormal state of health may show the same leucocyte percentages as a healthy person. Use test results in conjunction with clinical and other diagnostic data.
11. Certain patients may present special problems due to altered or very low numbers of certain cellular populations.
12. Patients with chronic HIV or elevated viemia may exhibit lower than expected NK lymphocyte results due to a phenotypic shift in the NK cell subsets. In these clinical conditions there is a selective loss of CD56+/CD16+ NK cell subset and an expansion of other pathologic NK cell populations. Use test results in conjunction with clinical and other diagnostic data.
13. Results obtained with flow cytometry may be erroneous if the laser is misaligned or the gates and regions are improperly set.
14. In some specimens, purity of the lymphocyte region may be decreased due to non-lymphoid contaminants with low SS and high CD45 fluorescence similar to lymphocyte populations. These samples may meet CD3+ Reliability Check acceptance criteria, as the relative proportion of CD3+CD4+ and CD3+CD8+ cells remains constant, yet the result may not be accurate. A review of all data plots for the presence of the expected staining patterns is recommended for all samples.
15. Performance has not been established for pediatric use.

PERFORMANCE CHARACTERISTICS
Refer to the AQUIOS Tetra System Guide PN B26364 for information on Reference Ranges, Linearity, Accuracy of Method, Precision, Analytical Measuring Ranges and Quality Control.

SPECIFICITY
The CD45 antigen is expressed on every type of hematopoietic cell except mature erythrocytes and their immediate progenitors. It has not been detected in differentiated nonhematopoietic tissue. The CD3 antigen is normally present on the cell surface of mature thymocytes and resting and activated peripheral blood mature T lymphocytes (both inducer and suppressor/cytotoxic populations). The CD4 antigen is present on thymocytes and the inducer T lymphocyte population in peripheral blood. It is also expressed at low density on monocytes.
The CD8 antigen is normally present on approximately 80% of thymocytes and approximately 30-35% of peripheral blood T lymphocytes and some natural killer cells.
The CD16 antigen is the low-affinity receptor for IgG (FcγRIII) that binds immune complexes, but not monomeric IgG. The CD16 antigen exists in two different forms encoded by two different genes: FcγRIIa (or II-3) and FcγRIIB (or II-1). The genetic heterogeneity of CD16 generates alternative membrane-anchored molecules. One is a transmembrane form (FcγRIIA, 50 – 65 kDa) expressed on NK cells, monocytes and macrophages. The other is a glycosylphosphatidylinositol (GPI)-anchored form (FcγRIIB, 48 kDa) only expressed on neutrophils. It has been shown that the CD16 antigen can be non-covalently associated within the membrane of NK cells, to the 16 kDa CD3ζ chain, or to the dimeric FcγRI chain. The 3G8 monoclonal antibody (mAb) binds to FcγRIIb as well as to FcγRIIa (strongly). It was shown to block almost completely the binding of IgG dimers to FcγRIIb. Experiments where amino acid mutations were made to the FcγRIIb molecule showed that the 3G8 mAb is affected by Lys102 and Val164 substitutions in the FG loop of the membrane-proximal Ig-like domain of the molecule. The 3G8 mAb has been assigned to the CD16 cluster of differentiation at the Fifth International Workshop on Human Leucocyte Differentiation Antigens held in Boston, USA, in 1993.
The CD19 antigen is expressed on all B cells, including early progenitor B cells. It can also be found on follicular dendritic cells and myelomonocytic lineage progenitor cells, but is not expressed on T cells, monocytes or granulocytes.
The CD56 antigen is expressed on a subpopulation of lymphocytes that demonstrate natural killer (NK) activity (and also on various types of non-circulating cells of neural and/or neuroendocrine origin). This subpopulation consists of both natural killer cells (CD56+CD16+) and a subset of T cells (CD3-CD56+). CD3-CD56+ cells are capable of mediating non-TCR mediated cytotoxicity in peripheral blood. CD56 is not expressed on other T or B lymphocyte, monocyte, granulocyte or erythrocyte populations.
The antigen specificity of the CD45, CD3, CD4, and CD8 monoclonal antibodies comprising the AQUIOS Tetra-1 Panel CD45-FITC/CD4-PE/CD8-PC5 and AQUIOS Tetra-2+ Panel CD45-FITC/CD56-PE/CD16-APC and CD16-APC/CD8-PC5 monoclonal antibody reagents have been previously established by the First, Fourth and Fifth International Workshops for Leukocyte Typing.
The antigen specificity of the CD45, CD3, CD8, CD16, and CD56 monoclonal antibodies comprising the AQUIOS Tetra-1 Panel CD45-FITC/CD4-PE/CD8-PC5 and AQUIOS Tetra-2+ Panel CD45-FITC/CD56-PE/CD16-APC and CD16-APC/CD8-PC5 monoclonal antibody reagents were screened on normal human adult donor blood samples. Results consistently demonstrated that the CD3, CD4, CD8, CD16, and CD56 (CD56-CD16)-1RD1 antibodies reacted specifically with the appropriate lymphocyte populations. Monoclonals were dirty stained with CD34 monoclonal antibody.

REFERENCES


46. Zloza, et al. Multiple populations of T lymphocytes are distinguished by the level of CD4 and CD8 coexpression and require individual consideration. Journal of Leukocyte Biology 2006:79(4-6).


49. EP09-A2 CLSI: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline.


**PRODUCT AVAILABILITY**

AQUIOS Tetra-1 Panel
CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5
B23533 - 50 tests (0.9 mL)

AQUIOS Tetra-2+ Panel
CD45-FITC/(CD56+CD16)-RD1/CD19-ECD/CD3-PC5
B23534 - 50 tests (0.9 mL)

**TRADEMARKS**

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For additional information, or if damaged product is received, call Beckman Coulter Customer Service at 800-742-2345 (USA or Canada) or contact your local Beckman Coulter Representative.

Glossary of Symbols is available at techdocs.beckmancoulter.com (PN C05838).

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**Revision History**

**Revision AC, 4/2015**

Changes were made to:
- Intended Use
- Summary and Explanation
- Clinical Relevance
- Storage Conditions and Stability
- Limitations
- Specificity
- References

**Revision AD, 08/2015**

Changes were made to:
- Add Romanian

**Revision AE, 06/2016**

Changes were made to:
- Add new languages
- Specimen Collection
- References