AQUIOS Tetra-1 Panel
CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5

AQUIOS Tetra-2+ Panel
(CD56+CD16)-RD1/CD19-ECD/CD3-PC5

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.\(^6\) CD45-assisted Leukocyte gating along with CD4 allow ready enumeration of absolute count and percentages of CD4 T cells.\(^2,3,4\) The expression of CD45 density is useful for discriminating between normal and malignant leukocytes. 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.\(^4\) These cell types are morphologically indistinguishable 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 and appear to play an integral part in regulation of hematopoiesis, the defense against viral infection, and the destruction of malignant tumor cells. The NK lymphocyte mediated cytolytic activity occurs without restriction by class I or II major histocompatibility complex (MHC) antigens.\(^5\)

CLINICAL RELEVANCE
CD3+, CD4+, CD8+, and/or CD19+ Lymphocytes
CD3+, CD4+, CD8+, and/or CD19+ lymphocyte percentages and absolute counts may be used as aids to evaluate immune competency underlying known or unknown disease states and to monitor lymphocyte levels following organ transplantation.\(^7,25,26,27,28,29,30,31,32,33\)

To illustrate, identification of abnormal levels of CD3+, CD4+, CD8+, and/or CD19+ lymphocytes may aid in the diagnosis and/or prognosis of unidentified disease conditions in patients with low white blood cell counts. Altered percentages of CD3+, CD4+, CD8+, and/or CD19+ lymphocytes recorded following organ (kidney, heart, liver, lung) transplantation suggest T (CD3+, CD4+, CD8+) and/or B (CD19+) lymphocyte measurements may be useful as aids in monitoring these cellular populations.

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 cytoxicity against targets such as certain tumor and virus-infected cells.\(^25\)

CD4/CD8 Ratio
Disease-related changes in CD4+ and/or 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.\(^17,36\) 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.\(^31,32\)

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\(^11,12,10\) 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-2+ 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:

$$\text{Total Lymphocyte Percentage} = \%\text{CD3} + \%\text{CD19} + \%\text{CD56} + \%\text{CD16}$$

Used as a panel, AQUIOS Tetra-1 Panel also functions for CD45-FITC/CD56-RD1/CD16-RD1/CD19-ECD/CD3-PC5 and 1.445/0.022/0.091/0.247/0.365 μg/test.

**REAGENT CONTENTS**

See table on page 1.

**STORAGE CONDITIONS AND STABILITY**

Unopened reagent is stable to the expiration date on the vial label. 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**

- May produce an allergic reaction.
- Denotes that the Safety Data Sheet is available at techdocs.beckmancoulter.com.

1. These reagents contain 0.1% sodium azide. Sodium azide under acid 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. These reagents contain 0.1% sodium azide. Sodium azide under acid 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.

3. These reagents contain 0.1% sodium azide. Sodium azide under acid 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.

4. These reagents contain 0.1% sodium azide. Sodium azide under acid 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.

5. These reagents contain 0.1% sodium azide. Sodium azide under acid 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.

6. These reagents contain 0.1% sodium azide. Sodium azide under acid 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.

7. These reagents contain 0.1% sodium azide. Sodium azide under acid 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.

8. These reagents contain 0.1% sodium azide. Sodium azide under acid 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.

9. These reagents contain 0.1% sodium azide. Sodium azide under acid 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.

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

**MATERIAL SUPPLIED**

- AQUIOS Tetra-1 Panel
  - B23533 – 50 tests (0.9 mL)
- AQUIOS Tetra-2+ Panel
  - B23534 – 50 tests (0.9 mL)
- B301/4 – Cap with Septa (1)

**MATERIALS REQUIRED BUT NOT SUPPLIED**

- AQUIOS Lysing Reagent Kit
- AQUIOS IMMUNO-TROL Low Cells, PN B26364
- MEMbroth
- EDTA
- Ficoll
- Stock solution 10× PBS
- Towels
- Gloves
- AQUIOS CL Flow Cytometer

**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.

**NOTE:** Once Cap with Septa is placed on vial do not remove.

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.

**EVIDENCE OF DETERIORATION**

Any change in the physical appearance of these reagents (normal appearance is a clear, pink liquid) or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used.
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) for detailed instructions.

LIMITATIONS

1. The reagents are for use only on AQUIOS Flow Cytometers.
2. Reagents 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 monoclonal 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. 41, 42, 43
10. Abnormal states of health are not always represented by abnormal percentages of certain leukocyte populations. An individual in an abnormal state of health may show the same leukocyte 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 viremia 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. 44, 45
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. 7, 8, 9, 10 It has not been detected in differentiated nonhematopoietic tissue. 7, 8, 9, 10

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). 10, 11, 12, 13, 14

The CD4 antigen is present on thymocytes and the inducer T lymphocyte population in peripheral blood. 13, 14 It is also expressed at low density on monocytes. 13, 14

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. 15, 16 The CD16 antigen is the low-affinity receptor for IgG (FcγRIIa) 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 FcγRIIib (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 (FcγRIIib, 48 kDa) only expressed on neutrophils. 10, 13 It has been shown that the CD16 antigen can be non-covalently associated within the membrane of NK cells, to the 16 kDa CD3δ chain, 17 or to the dimeric FcγR chain. 18 The 3G8 monoclonal antibody (mAb) binds to FcγRIIa as well as to FcγRIIib (strongly). It was shown to block almost completely the binding of IgG dimers to FcγRIIib. 19 Experiments where amino acid mutations were made to the FcγRIIib 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. 17, 19 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. 20

The CD19 antigen is expressed on all B cells, including early progenitor B cells. 21 It can also be found on follicular dendritic cells and myelomonocytic lineage progenitor cells, but is not expressed on T cells, monocytes or granulocytes. 10, 11, 12, 13

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). 22 This subpopulation consists of both natural killer cells (CD56+CD3−) and a subset of T cells (CD56+CD3+). 13, 19, 21, 22 CD56+ cells are capable of mediating non-TCR mediated cytolytic activity in peripheral blood. 21, 22 CD56 is not expressed on other T or B lymphocyte, monocyte, granulocyte or erythrocyte populations. 13, 24, 25

The antigen specificity of the CD45, CD3, CD4, and CD8 monoclonal antibodies comprising the AQUIOS Tetra-1 Panel CD45-FITC/CD4-PE/CD8-PerCP, CD3-phycoerythrin in AQUIOS Tetra2+ Panel CD45-FITC/CD25-APC/CD71-PECy7 and AQUIOS Tetra-1 Panel CD45-FITC/CD69-APC/CD14-PEy7/CD11c-PEy7 monoclonal antibody reagents has been previously established by the First, Fourth and Fifth International Workshops for Leukocyte Typing. 1, 2

The antigen specificity of the CD45, CD3, CD19, CD56 and CD16 monoclonal antibodies comprising the AQUIOS Tetra-2+ Panel CD45-FITC/CD56-APC/CD16-PerCP and AQUIOS Tetra-1 Panel CD45-FITC/CD69-APC/CD14-PEy7/CD11c-PEy7 monoclonal antibody reagents has been previously established by the First, Fourth and Fifth International Workshops for Leukocyte Typing. 1, 2

To assess cellular cross-reactivity, the CD3, CD4, CD8, CD19, and CD56 monoclonal antibodies comprising the AQUIOS Tetra-1 Panel CD45-FITC/CD4-RD1/CD8-ECD/CD3-PC5 and AQUIOS Tetra-2+ Panel CD45-FITC/CD56-APC/CD16-PerCP/CD71-PECy7 monoclonal antibody reagents were screened on normal human adult donor blood samples. Results consistently demonstrated that the CD3, CD4, CD8, CD19, and CD56 (CD56-CD16)-RD1 antibodies react specifically with the appropriate lymphocyte populations. Monocytes were dimly stained with CD4 monochlonal antibody.

REFERENCES


33. Ramos EL, Turka LA, Legatt JE, Wood IG, Milford EL and Carpenter CB. Decrease in phenotypically defined T helper inducer cells (T4+8B4+) and increase in T suppressor effector cells (T8+2H4+) in stable renal allograft recipients. Transplantation, 1989, 47:467-487.


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

Beckman Coulter, the stylized logo, and the Beckman Coulter product and service marks mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries.

Texas Red X is a trademark of Molecular Probes, Inc.

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).

© 2017 Beckman Coulter, Inc.
All Rights Reserved.

Revision History

Revision AD, 08/2015
Changes were made to:
- Add Romanian

Revision AE, 06/2016
Changes were made to:
- Add new languages
- Specimen Collection
- References

Revision AF, 09/2017
Changes were made to:
- Add new languages