Optimized for the Beckman Coulter Cytomics FC 500 and FC 500 MPL. The powerful dual laser FC 500 flow cytometer easily accommodates the Immunotox Three-color (iTox3) Combinations. For deeper investigation into the immune status, the FC 500 Series offers simplified five-color analysis, implementing the easiest and most advanced method of color compensation, the Advanced Digital Compensation (ADC) method.

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### Ordering Information

**Beckman Coulter IOTest® Anti-Rat CD3-FITC / CD4-PC7 / CD8-APC**

- **Part Number:** A32909
- **Size:** 50 tests
- **Use:** 25 µL/test
- **Regulatory Status:** RUO

**Beckman Coulter IOTest® Anti-Rat CD3-FITC / CD45RA-PC7 / CD161a-APC**

- **Part Number:** A32910
- **Size:** 50 tests
- **Use:** 25 µL/test
- **Regulatory Status:** RUO

**Complementary Reagents**

- **Clone:** 1F4
- **Line:** OX-38
- **Size:** 50 tests
- **Part Number:** IM3422 or A07704

- **Clone:** 10/78
- **Line:** OX-33
- **Size:** 50 tests
- **Part Number:** IM3648 or A09777

- **Clone:** OX-1
- **Line:** IOTest 3 Fixative Solution (8% paraformaldehyde)
- **Size:** 50 tests
- **Part Number:** IM3515 or A07800

**Automate Sample Preparation utilizing the Beckman Coulter Biomek® NXP**

- **Biomek NXP sets a new standard for flexible laboratory solutions.**
- **Liquid handling - including pipetting, dilution, dispensing, and combining samples with reagents - into a single, small-footprint that's as powerful and flexible as it is efficient and economical.**

**Biological Samples and Procedure:**

The Beckman Coulter Immunotox Three-color Combinations are suitable with a number of biological samples, including splenocytes, bone marrow, and whole blood. The samples are lysed with the Beckman Coulter VersaLyse Lysing Solution, implemented in its simultaneous "Fix-and-Lyse" procedure by the addition of 0.2% of paraformaldehyde.

- **Dispense:** 25 µL iTox3 Anti-Rat combination
- **Dispense:** 25 µL Rat biological specimen
- **Vortex:** Incubate 20 minutes at room T°, protected from light
- **Add:** 2 µL "Fix-and-Lyse" solution
- **Vortex:** Incubate 10 minutes at room T°, protected from light
- **Analysis:** without delay! or keep at 2-8°C, protected from light, and analyze within 2 hours.

Caution: these reagents are designed for use with certain rat biological samples and are not to be used with human biological samples. For Research Use Only.
Rapid and accurate enumeration of Rat T-, B-, and NK-cell populations

No-wash procedure

Comparable with cell viability assessment

Activation marker-ready

Easy to implement manually

Automation-ready

Immunotoxicity Overview:
Immunotoxicology is the study of injury to, or injury caused by the immune system often resulting from exposure to environmental chemicals, pharmaceutical, or biological materials. During pre-clinical drug discovery studies immunotoxicology evaluations in animal models are necessary. Lymphocyte subset immunophenotyping of rat biological samples is an example of the recommended tests.

Choice of Markers:

CD3, CD4, CD8:
T cells help to coordinate cell mediated and humoral immune responses. They can be distinguished from other lymphocytes based on their expression of the T-cell receptor (TCR), and its associated invariant CD3 complex, which is involved in TCR signal transduction. CD3-specific monoclonal antibodies (mAbs) are well suited as markers for mature T cells. Subsets of mature T cells can be specified by the expression of CD4 or CD8 molecules, the two major markers characterizing helper and suppressor T cells, respectively.

CD45RA:
This marker is used to identify mature B cells (in the mature B-cell subset the CD45RA+ population is positive, whereas the CD45RA-negative population is negative). The B cells generated during the T-cell maturation process are positive for CD45RA. Amongst these B-cell isoforms of CD45, the BCD ITOX application can be distinguished from other lymphocytes on the basis of their expression of CD45 isoforms. Among these B-cell isoforms of CD45, the BCD ITOX application targets CD45RA, as recognised by OX-33 mAb.

CD161a (NKR-P1A):
NK cells are the third population of lymphocytes, not expressing either a TCR or a BCR (B-cell receptor). NK cells are capable of antibody-dependent cell mediated cytotoxicity, as well as cytotoxicity without prior sensitization or coating of the target cell with antibody. NK cells in the rat can be distinguished from other lymphocytes on the high level expression of CD161a (NKR-P1A). The majority of NK cells express CD8a, but do not co-express CD3.

Tested Quality

Like all other Beckman Coulter products, the Immunotox Three-color (iTox3) Combinations have been carefully standardized and validated to provide you with accurate and reproducible results. This is true when implementing a no-wash red blood cell lysing procedure, as well as if you decide to wash your samples before flow cytometry analysis. The following examples, all tested with the VersaLyse™ “Fix-and-Lyse” procedure without a wash, show the tremendous discrimination obtained on different specimen types. This set of examples also demonstrates different gating strategies adapted to the chosen analyzed parameters.

Figure 1: Analysis of a Wistar Rat whole blood sample. Immunophenotyping is with reagent # A32909 (first and second histograms, featuring red and black dots), combining CD3-FITC (clone 1F4), CD4-PC7 (clone OX-38), and CD8-APC (clone OX-8). Figure 2: Analysis of a Wistar Rat bone marrow sample. Immunophenotyping is with reagent # A32909, combining CD3-FITC (clone 1F4), CD45RA-APC (clone OX-33), and CD161a-APC (clone 10/78). As a second drop-in example, 7-AAD Viability Dye is added to discriminate dead from viable cells.

A preliminary analysis step implements standard light scatter gating of the lymphocytes and a Forward Scatter (FS) vs. Side Scatter (SS) histogram (not shown). Then the gating strategy mainly relies on the discriminative power of CD3. In the first histogram (lower left) T cells are identified by a CD3-positive gate within Gate A, for further CD4-positive (Gate C) or CD8-positive (Gate D) T-cell subpopulation analysis in the CD8 vs. CD4 histogram (upper right). In the third histogram (lower left), CD4-exclusion (Gate F) is realized within Gate B, for further CD45RA-positive (Gate G) or CD161a-positive (Gate H) T-cell subpopulation analysis, respectively, and performed in the CD3 vs. CD45RA histogram (lower right).
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Beckman Coulter offers a standardized and comprehensive flow cytometry application to address lymphocyte subset immunophenotyping. By design, this methodology aims at streamlining the preparation steps, with easy procedures, adapted to several different biological samples, whatever the lymphocyte sub-family level you want to reach. Providing the flexibility to automate the process or to manually perform your testing with the same high level of confidence in results that you expect from Beckman Coulter.

Choice of Markers:

CD3, CD4, CD8:

T cells help to coordinate cell mediated and humoral immune responses. They can be distinguished from other lymphocytes based on their expression of the T-cell receptor (TCR), and its associated invariant CD3 complex, which is involved in TCR signal transduction. CD3-specific monoclonal antibodies (mAb) are well suited as markers for mature T Cells. Subsets of mature T cells can be specified by the expression of CD4 or CD8 molecules, the two major markers characterizing helper and suppressor T cells, respectively.

CD45RA:

It discriminates different gating strategies adapted to the chosen analyzed parameters. A preliminary analysis step implements standard light scatter gating of the lymphocytes on a Forward Scatter (FS) vs. Side Scatter (SS) histogram (not shown). Then the gating strategy mainly relies on the discriminative power of CD3. In the first histogram (upper left), CD45RA-positive (Gate F) or CD161a-positive (Gate G) T cells are distinguished within Gate B, for further CD4-positive (Gate C) or CD8-positive (Gate D) T-cell subpopulation analysis in the CD8 vs. CD4 histogram (upper right). In the third histogram (lower left), CD3-exclusion (Gate E) vs. CD4+ and CD8+ T cells are distinguished within Gate A. gate J: Viable CD45RA+ B cells

cursor I: viable CD161a+ cells
cursor H: Viable CD45RA+ cells
cursor G: Viable CD3+ T cells

gate F: viable Splenocytes
gate E: Splenocytes

cursor D: CD3+ T cells

cursor C: CD3+ T cells

cursor B: CD3- T cells

cursor A: lymphocytes

gate D: CD4-CD8+ double positive T cells

gate C: CD4+CD8- single positive T cells

gate B: CD4-CD8- double negative T cells

gate A: lymphocytes

Figure 1: Analysis of a Wistar Rat whole blood sample. Immunophenotyping is with reagent # A32909 (first and second histograms, featuring red and black dots), combining CD3-FITC (clone 1F4), CD4-PC7 (clone OX-38), and CD8-APC (clone OX-8), and a no-wash red blood cell lysing procedure, as well as if you decide to wash your samples before flow cytometry analysis. The following examples, all lysed with the VersaLyse™ “Fix-and-Lyse” procedure without a wash, show the tremendous discrimination obtained on different specimen types. This set of examples also demonstrates different 2-color gating strategies adapted to the chosen analyzed parameters.

Figure 2: Analysis of a Wistar Rat bone marrow sample. Immunophenotyping is with reagent # A32909 and a no-wash red blood cell lysing procedure, as well as if you decide to wash your samples before flow cytometry analysis. The following examples, all lysed with the VersaLyse™ “Fix-and-Lyse” procedure without a wash, show the tremendous discrimination obtained on different specimen types. This set of examples also demonstrates different 2-color gating strategies adapted to the chosen analyzed parameters.

Figure 3: Analysis of a Wistar Rat spleen sample. Immunophenotyping is with reagent # A32909, combining CD3-FITC (clone 1F4), CD45RA-PC7 (clone OX-33), and CD161a-APC (clone 10/78). As a drop-in example, 7-AAD Viability Dye is added to discriminate dead from viable cells.

Figure 4: Analysis of a Wistar Rat whole blood sample. Immunophenotyping is with reagent # A32909. As a second drop-in example, anti-Rat CD45-PE is added for lymphocyte gating purposes.
Rapid and accurate enumeration of Rat T-, B-, and NK-cell populations

No-wash procedure

- Automation-ready

Choice of Markers:

- CD3, CD4, CD8: T-cells help to coordinate cell-mediated and humoral immune responses. They can be distinguished from other lymphocytes based on their expression of the T-cell receptor (TCR), and its associated invariant CD3 complex, which is involved in TCR signal transduction. CD3-specific monoclonal antibodies (mAb) are well suited as activation marker-ready.

- CD45RA: mature T cells can be distinguished from other lymphocytes on the basis of their expression of CD45RA. Targets CD45RA, as recognized by OX-33 mAb.

- CD161a (NKR-P1A): NK cells can be distinguished from other lymphocytes based on the expression of CD161a (NKR-P1A). The majority of NK cells express CD161a, but do not co-express CD3.

- CD45RA-PE (clone OX-33) anti-Rat CD45-PE is added for lymphocyte gating purposes.

- CD161a-APC (clone 10/78): as a drop-in example, 7-AAD Viability Dye is added to discriminate dead from viable cells.

Training Quality

- Gate A: Lymphocytes (structure vs. size gating)
- Gate B: Lymphocytes (structure vs. CD3 gating)
- Gate C: CD3+ T cells
- Gate D: CD8+ T cells
- Gate E: CD45RA+ B cells
- Gate F: CD161a+ NK cells

- Gate G: CD161a+ NK cells
- Gate H: Viable CD45RA+ cells
- Gate I: Viable CD161a+ cells
- Gate J: Viable CD45RA+ B cells
- Gate K: Viable CD161a+ NK cells

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- Immunoassays

- automated

- manual

- Activation marker-ready

- Easy to implement manually

- Automation-ready

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Beckman Coulter IOTest® Anti-Rat CD3-FITC / CD4-PC7 / CD8-APC

- Rat T-Cell Subpopulation iTox3 Combination
- Part Number: A32909
- Size: 50 tests
- Use: 25 µL/test
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Beckman Coulter IOTest® Anti-Rat CD3-FITC / CD45RA-PC7 / CD161a-APC

- Rat T/B/NK Cell Population iTox3 Combination
- Part Number: A32910
- Size: 50 tests
- Use: 25 µL/test
- Regulatory Status: RUO

Complementary Reagents
- Clone: Line Size Part Number
- Anti-Rat CD45-PE OX-1 IOTest® 50 tests A36700 (soon available)
- 7-AAD Viability Dye - - 150 tests IM3422 or A07704
- VersaLyse™ Lysing Solution - - 100 tests IM3648 or A09777
- IOTest 3 Fixative Solution (8% paraformaldehyde) - - 100 tests IM3515 or A07800

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Biological Samples and Procedure:
The Beckman Coulter IMMUNOTOX Three-color Combinations are suitable with a number of biological samples, including splenocytes, bone marrow, and whole blood. The procedure is as follows:

1. Dispense: 25 µL iTox3 Anti-Rat combination
2. Incubate 20 minutes at room T°, protected from light
3. Dispense: 25 µL Rat biological specimen
4. Incubate 10 minutes at room T°, protected from light
5. Analyze...

Automate Sample Loading using the Beckman Coulter Multi-Platform Loader (MPL) option of the FC 500 Series provides the capability to automate sample loading using microtubes (e.g. Micronic, Ref. M32022) arranged on a 96-well rack. It can also take on a 40-tube rack for standard 12 x 75 mm test tubes.

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<td></td>
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Complementary Reagents

Anti-Rat CD45-PE
Species: Mouse anti-Rat
Isotype: IgM
Clone: OX-1
Complementary Reagents: IOTest 3 Fixative Solution (8% paraformaldehyde)

CD3 CD45RA CD161a

Fluorescence

Beckman Coulter 488 nm 635 nm
Cytomics FC 500

FC 500 Optical Channels
FL1 FL2 FL3 FL4

FC 500 MPL Optical Channels
FL1 FL2 FL3 FL4

FC 500 MPL Optical Channels
D5C

FL5 APC

Combination #1
CD3 CD4 CD8

Combination #2
CD3 CD45RA CD161a

Gating Tool CD45-PE

Activation Marker Example CD25-PE

Viability Dye 7-AAD

Strengthen, Automation-Ready Immunotoxicology Reagents for Flexibility in Rat Lymphocyte Phenotyping

IMMUNOTOX THREE-COLOR COMBINATIONS: iTox3

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