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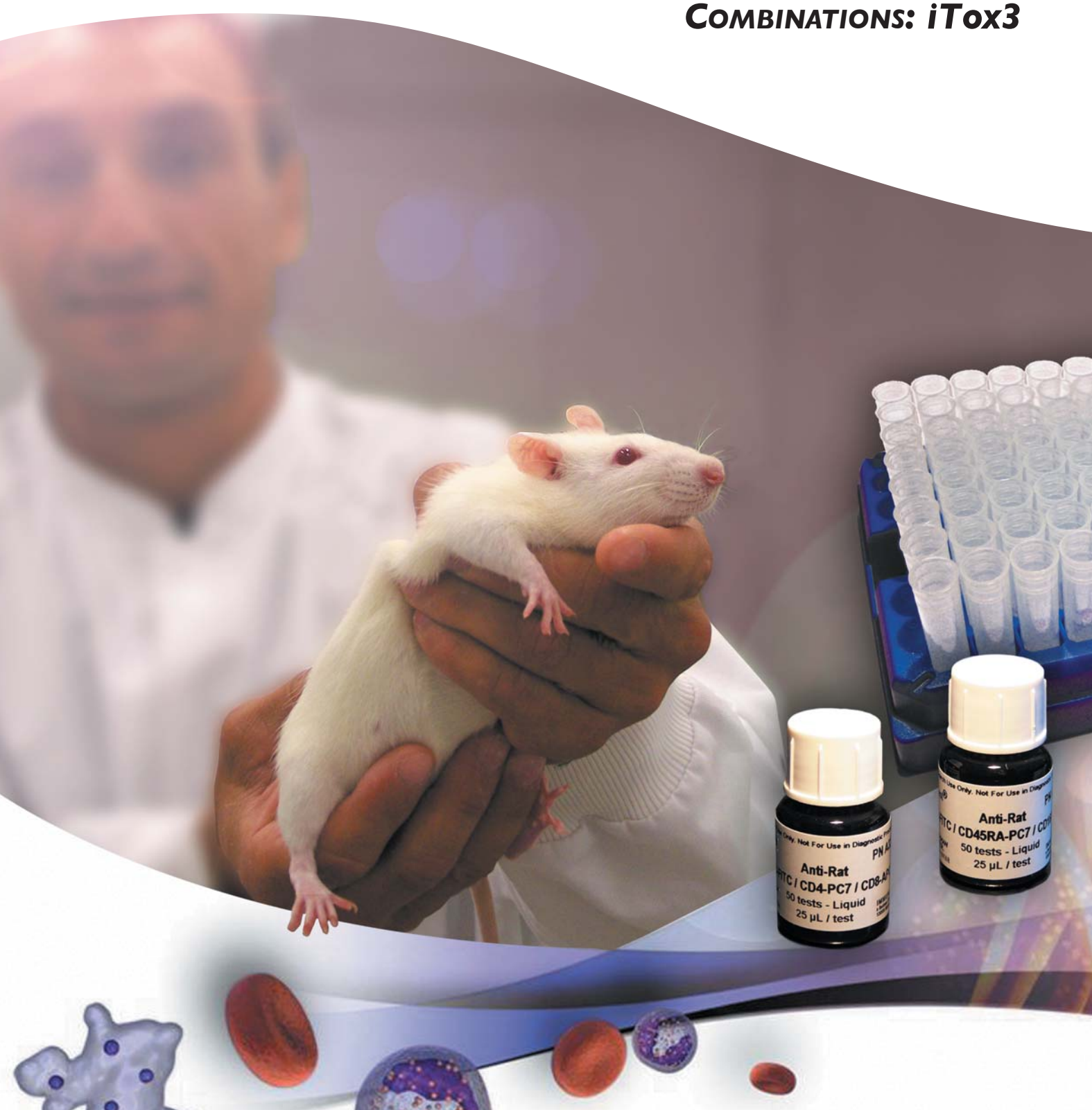
BECKMAN COULTER®

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

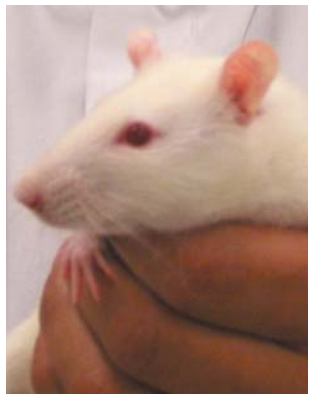
IMMUNOTOX THREE-COLOR COMBINATIONS: iTox3



- *Rapid and accurate enumeration of Rat T-, B-, and NK-cell populations Including $CD4^+$ and $CD8^+$ T-cell sub-populations*
- *No-wash procedure*
- *Compatible with cell-viability assessment*
- *Activation marker-ready*
- *Easy to implement manually*
- *Automation-ready*

Immunotoxicology Overview:

Immunotoxicology is the study of injury to, or injury caused by the immune system often resulting from exposure to environmental chemicals, pharmaceutical, or biologic 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.



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.

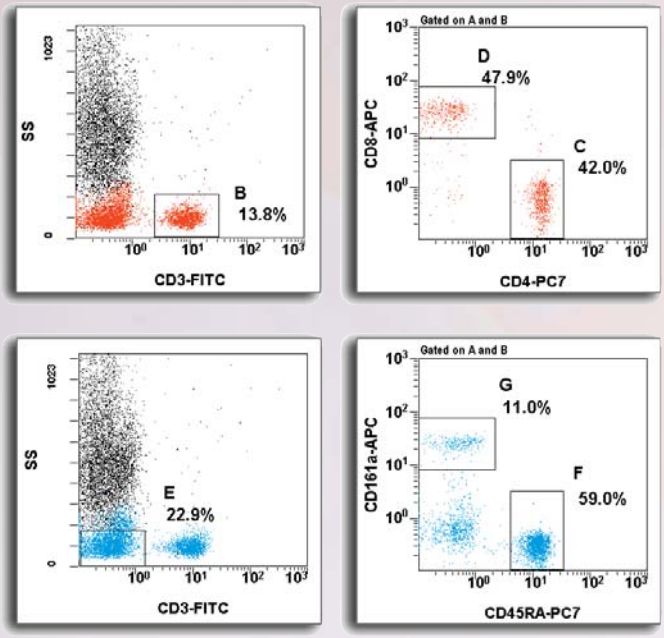
CD45RA:
B cells generate the humoral immune response and belong to the antigen presenting cells (APCs) that are necessary for T cell maturation. Mature B cells in the rat can be distinguished from other lymphocytes on the basis of their expression of CD45 isoforms. Amongst these B-cell isoforms of CD45, the BCI ITOX application targets CD45RA, as recognized 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 by 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 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 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), and with reagent #A32910 (third and fourth histograms, featuring blue and black dots), combining CD3-FITC (clone 1F4), CD45RA-PC7 (clone OX-33), and CD161a-APC (clone 10/78).



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), **CD3-inclusion** of T cells is realized 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** of T cells is realized within Gate E. For further CD45RA-positive (Gate F) or CD161a-positive (Gate G) B-cell and NK-cell population analysis, respectively, are performed in the CD161a *vs.* CD45RA histogram (lower right).

Gate A: Lymphocytes
Gate B: $CD3^+$ T cells
Gate C: $CD4^+$ T cells
Gate D: $CD8^+$ T cells
Gate E: $CD3^-$ non-T cells
Gate F: $CD45RA^+$ B cells
Gate G: $CD161a^+$ NK cells

Figure 2: Analysis of a Wistar Rat bone marrow sample. Immunophenotyping is with reagent # A32909, combining CD3-FITC (clone 1F4), CD4-PC7 (clone OX-38), and CD8-APC (clone OX-8).

Gate A: Lymphocytes
Cursor B: $CD3^+$ T cells
Gate C: $CD3^+CD4^+$ double positive T cells
Gate D: $CD3^+CD8^+$ double positive T cells

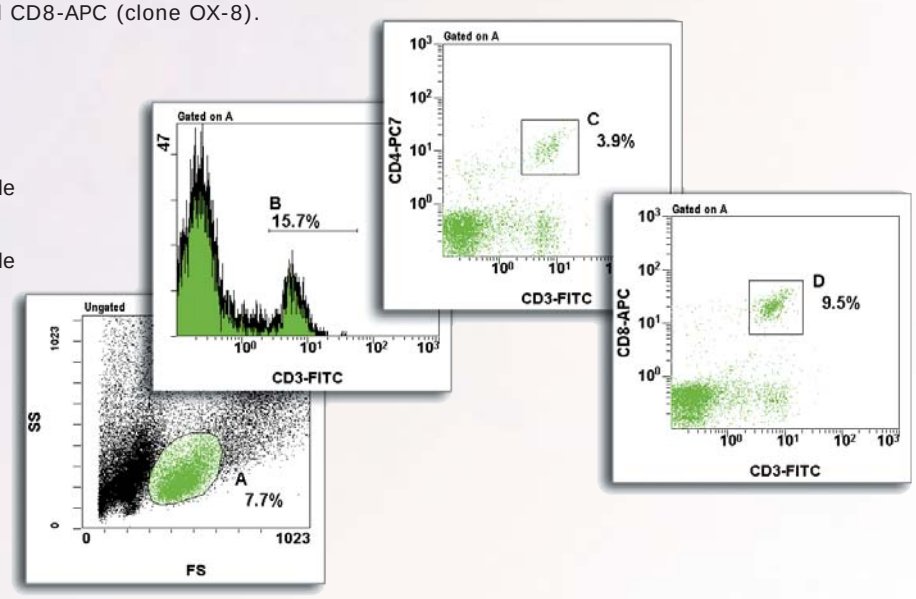
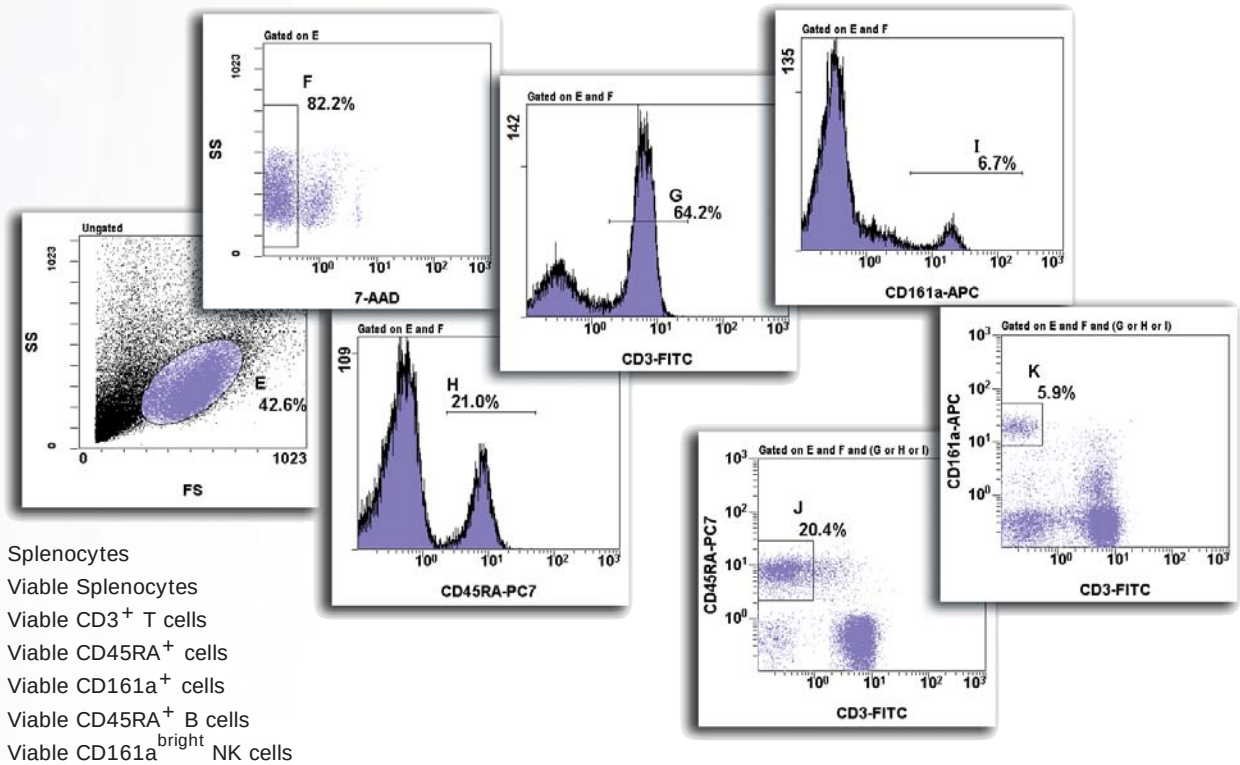
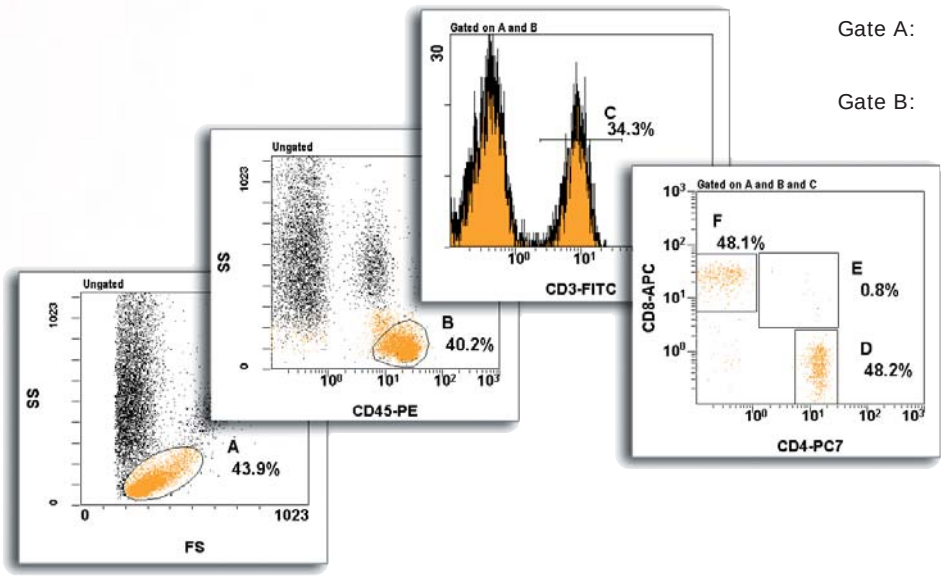


Figure 3: Analysis of a Wistar Rat spleen sample. Immunophenotyping is with reagent # A32910, combining CD3-FITC (clone 1F4), CD45RA-PC7 (clone OX-33), and CD161a-APC (clone 10/78). As a drop-in example, 7-AAD Viability Dye is added to discriminate dead from viable cells.



Gate E: Splenocytes
Gate F: Viable Splenocytes
Cursor G: Viable $CD3^+$ T cells
Cursor H: Viable $CD45RA^+$ cells
Cursor I: Viable $CD161a^+$ cells
Gate J: Viable $CD45RA^+$ B cells
Gate K: Viable $CD161a^{bright}$ NK 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.



Gate A: Lymphocytes (structure *vs.* size gating)

Gate B: Lymphocytes (structure *vs.* CD45 gating)

Cursor C: $CD3^+$ T cells

Gate D: $CD45^+CD3^+CD4^+$ T cells

Gate E: $CD45^+CD3^+CD4^+CD8^+$ "double positive" T cells

Gate F: $CD45^+CD3^+CD8^+$ T cells

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.

Beckman Coulter Cytomics FC 500 Excitation	488 nm Argon Laser				635 nm HeNe Laser
FC 500 Optical Channels	FL1	FL2	FL3	FL5	FL4
	FITC	OPEN		PC7	APC
Combination #1	CD3	SLOTS		CD4	CD8
Combination #2	CD3			CD45RA	CD161a
Drop-in Examples: Gating Tool	CD45-PE				
Drop-in Examples: Activation Marker Example	CD25-PE				
Drop-in Examples: Viability Dye	7-AAD				

Table 1: The design of the two Immunotox combinations simplifies the compensation in the basic three-color configurations (FITC / PC7 / APC) and it allows the option of adding extra markers to customize the analysis in a flexible way.

The 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.

Automate Sample Preparation utilizing the Beckman Coulter Biomek® NXP

The Biomek NXP sets a new standard for flexible laboratory solutions. It puts every aspect of 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. All these samples are ideally 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:	1 mL "Fix-and-Lyse" solution
	Vortex
	Incubate 10 minutes at room T°, protected from light
Analyze...	without delay! or keep at 2-8°C, protected from light, and analyze within 2 hours.

Ordering Information

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
Regulatory Status: RUO

	CD3	CD4	CD8
Clones	1F4	OX-38	OX-8
Species	Mouse anti-Rat	Mouse anti-Rat	Mouse anti-Rat
Isotypes	IgM	IgG2a	IgG1

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

	CD3	CD45RA	CD161a
Clones	1F4	OX-33	10/78
Species	Mouse anti-Rat	Mouse anti-Rat	Mouse anti-Rat
Isotypes	IgM	IgG1	IgG1

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

Caution: these reagents are designed for use with certain rat biological samples and are not to be used with human biological samples.

For Reseach Use Only

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B2006-7320

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