**DuraClone IM Treg Tube, 25 Tests, RUO**

**REF B53346 – 25 tests**

IFU- B53346-1.0

BECKMAN COULTER

| | Specifications of Constituent 1 | Specifications of Constituent 2 | Specifications of Constituent 3 | Specifications of Constituent 4 | Specifications of Constituent 5 | Specifications of Constituent 6 | Specifications of Constituent 7 | Specifications of Constituent 8 |
|---|---|---|---|---|---|---|---|---|
| **Specificity** | CD45RA | CD25 | CD39 | CD4 | FoxP3 | CD3 | Helios | CD45 |
| **Clone** | 2H4LDH11LDB9(2H4) | B1.49.9 | BA54 | SFCI12T4D11(T4) | 259D | UCHT-1 | 22F6 | J33 |
| **Immunogen** | T lymphocyte derived from Aotus trivirgatus | Human alloactivated T lymphocytes (FC2) | CD4+ CD8+ thymic clone B12 | Peripheral blood lymphocytes | human FOXP3 recombinant protein | T cell line + IL2 | Helios peptide (aa51-107) | Lazz 221 cell line |
| **Isotype** | IgG1 | IgG2a | IgG1 kappa | IgG1 | IgG1 kappa | IgG1 kappa | IgG | IgG1 kappa |
| **Species** | Mouse | Mouse | Mouse | Mouse | Mouse | Mouse | Hamster | Mouse |
| **Source** | Conditioned media | Ascites fluid or supernatant of in vitro cultured hybridoma cells | Supernatant | Conditioned media | Ascites fluid or supernatant of in vitro cultured hybridoma cells | Ascites fluid | Purified | Ascites fluid |
| **Purification** | Affinity chromatography | Affinity chromatography | Affinity chromatography | Affinity chromatography | Affinity chromatography | Ion exchange or affinity chromatography | Affinty chromatography | Affinity chromatography |
| **Fluorochrome** | Fluorescein isothiocyanate (FITC) | R Phycoerythrin (PE) | R Phycoerythrin-Cyanine 5.5 (PC5.5) | R Phycoerythrin-Cyanine 7 (PC7) | Alexa Fluor 647 (A647) | Allophycocyanin Alexa Fluor 750 (APC-A750) | Pacific Blue (PBE) | Krome Orange (KrO) |
| **λ Excitation** | 468-509 nm | 488 nm | 488 nm | 486-580 nm | 633/638 nm | 633 nm | 405 nm | 405 nm |
| **Emission peak** | 504-541 nm | 575 nm | 692 nm | 710-800 nm | 665 nm | 783 nm | 455 nm | 528 nm |

**For Research Use Only.**
**Not for use in diagnostic procedures.**

## BACKGROUND

Regulatory T cells (Tregs) play a crucial role in the induction and maintenance of immunological tolerance. Together with CD4 and CD25, the expression of the transcription factor FoxP3 is considered the hallmark of human regulatory T cells (CD3+CD4+ CD25+ FoxP3+).[1] Helios, a transcription factor of the *Ikaros* family, has been described as a marker identifying thymus-derived nTregs - as opposed to Tregs induced in peripheral tissues (iTregs). Additionally, Helios acts as an enhancer of regulatory function.[2]

Surface expression of ectoenzyme CD39 exerts a suppressive function by its ATPase activity and is restricted to an effector/memory-like subpopulation of Tregs.[3] CD45RA surface expression indicates a naïve or unprimed subpopulation of Tregs.[4]

## APPLICATION

The DuraClone IM Treg Tube, 25 Tests, RUO can be used to identify subpopulations of regulatory T cells in human whole blood samples by eight color flow cytometry.

## PRINCIPLE

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by T lymphocyte subpopulations. Specific surface staining of the T lymphocytes is performed by incubating the sample in the DuraClone IM Treg Tube 1.

The PerFix nc Buffer 1 is used to fix cells, Buffer 2 is used to induce permeability in the cytoplasmic and nuclear membranes of the T lymphocytes to enable staining of the intracellular determinants in the DuraClone IM Treg Tube 2. Upon permeabilization, the flow cytometer measures light diffusion and the fluorescence of cells; it enables the delimitation of the population of interest within an electronic gate defined on a bivariate histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) and the diffusion of narrow angle light (Forward Scatter or FS). Other bivariate histograms (dot plots) combining two out of the different parameters available on the cytometer can be used to position further electronic gates in order to select populations of interest for further analysis of respective light scattering and fluorescent parameters.

## KIT BOX CONTENTS

DuraClone IM Treg Tube, 25 Tests, RUO contains the following:

- 25 tests of the DuraClone IM Treg Tube 1
- 25 tests of the DuraClone IM Treg Tube 2
- 3 Compensation Kits containing:
  - CD4-FITC
  - CD4-PE
  - CD39-PC5.5
  - CD4-PC7
  - FoxP3-A647
  - CD3-APC-A750
  - CD4-PBE
  - CD8-Krome Orange

## STATEMENT OF WARNINGS

1. For stability information on DuraClone IM Treg tube, 25 Tests, RUO refer to the Certificate of Analysis (COA). Discard reagent or compensation tubes, as per applicable regulations.
2. Do not store the reagent or compensation tubes in the refrigerator; do not freeze/thaw the tubes.
3. All blood samples must be considered as potentially infectious and must be handled with care (protective gloves, gowns and goggles must be used while handling blood samples).
4. Discard reagent and compensation tubes containing processed samples, as per applicable regulations, after sample acquisition and analysis.
5. Minimize the exposure of the tubes to light, especially during incubation of sample(s) stained with fluorescent antibodies or during processing of sample(s), before acquisition.
6. Only calibrated instruments, as per the manufacturer's instructions, should be used.
7. Adhere to the instructions for use while using this reagent.
8. Seal the zip lock of the pouch containing reagent tubes after removing the desired number of tests.
9. Reagent and compensation tubes must be stored within the sealed pouch containing desiccant packs to prevent the tubes from being exposed to moisture.

## STORAGE CONDITIONS

Store the reagent and compensation tubes between 20 and 30°C, in a dry place and protected from direct exposure to light and moisture.

## EVIDENCE OF DETERIORATION

Any damage to the panel tube may indicate product deterioration and the product should not be used. Please contact your local distributor or you can contact Beckman Coulter at the following email address: duraclone-support@beckman.com

## INSTRUMENT REQUIREMENTS

This reagent is designed to be used on a flow cytometer capable of detecting forward and side scatter, and compatible with the excitation and emission spectra of the fluorochromes used in the reagent.

## SPECIMEN COLLECTION

The whole blood sample should be collected in a blood collection tube containing $K_2$EDTA. Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected. The sample must be stored between 20°C and 30°C.

## MATERIAL REQUIRED BUT NOT SUPPLIED

Blood collection tube containing $K_2$EDTA

Calibrated pipettes suitable for volumes from 5 to 1000ul

Vortex mixer

Sheath fluid

Flow-Check Pro Fluorospheres (REF. A69183) (For Navios/Gallios alignment verification)

Flow-Set Pro Fluorospheres (REF. A69184) (For Navios/Gallios standardization)

VersaComp Antibody Capture Beads Kit REF B22804) (For Compensation Setup)

PerFix-nc Kit (REF. B31168)-Containing PerFix nc Buffer 1 (Fixative Reagent), Buffer 2 (Permeabilizing Reagent) and Buffer 3 (Final 10X Solution)

Fetal Calf Serum (100%)

Phosphate Buffered Saline (PBS) (1X)

Flow cytometer

## PROCEDURE

### Sample Preparation

1. Add 50µL of fresh whole blood to DuraClone IM Treg Tube 1 and vortex at high speed for 6-8 seconds. Incubate Tube 1 for 15 minutes, protected from direct light exposure at 20 to 30°C.
2. Add 3 mL of 1XPBS to the tube and centrifuge at 500 x g for 5 minutes at 20 to 30°C.
3. Aspirate the supernatant. Gently vortex the cell pellet for 6-8 seconds. Re-suspend the cell pellet present in Tube 1 in 50 µL 100% fetal calf serum.
4. Add 5µL PerFix nc reagent Buffer 1 (Fixative Reagent) to the tube and vortex for 6-8 seconds.
5. Incubate Tube 1 for 15 minutes, protected from the direct light exposure at 20 to 30°C.
6. Add 400uL of PerFix nc Buffer 2 (Permeabilizing Reagent) to the Tube 1 and vortex for 6-8 seconds.
7. Transfer the contents of DuraClone IM Treg Tube 1 to DuraClone IM Treg Tube 2
8. Vortex Tube 2 for 6-8 seconds. Incubate Tube 2 for 60 minutes protected from the direct light exposure at 20 to 30°C.
9. Add 3 mL 1X PBS to Tube 2 and incubate the tube for 5 minutes.
10. Centrifuge Tube 2 at 500 x g for 5 minutes at 20 to 30°C.

11. Aspirate the supernatant. Gently vortex the cell pellet for 6-8 seconds. Re-suspend the cell pellet in 3 mL of 1X PerFix nc Buffer 3 (Buffer 3 is supplied at 10X and must be diluted in distilled water to prepare 1X solution).
12. Centrifuge the tube at 500 x g for 5 minutes at 20 to 30°C. Aspirate the supernatant. Gently vortex the cell pellet for 6-8 seconds. Re-suspend the cell pellet in 500 µL of 1X PerFix nc Buffer 3.
13. The sample is now ready for acquisition.

## COMPENSATION SETUP

1. Add 50 µL of fresh whole blood to each of the eight color compensation tubes from a single Compensation Kit provided in the DuraClone IM Treg Tube, 25 Tests, RUO box.
2. Add two drops of the positive VersaComp antibody capture beads to the following compensation tubes:
   a. CD39-PC5.5 compensation tube
   b. FoxP3-A647 compensation tube
3. Vortex all eight compensation tubes at high speed for 6-8 seconds and incubate the tube for 15 minutes, protected from the direct light exposure at 20 to 30ºC.
4. Process the blood samples in all eight compensation kit tubes by following steps 2-6 in the Sample Preparation procedure.
5. Do not follow step 7 in the Sample Preparation procedure and proceed with step 8 to 13 as listed in the Sample Preparation procedure.
6. For sample acquisition on Navios/Gallios:
   a. The AutoSetup Scheduler on the Navios groups selected applications for efficient sampling from common compensation samples when scheduling multiple applications and provides the carousel load report to facilitate setting up and loading samples for daily QC. For setting up compensation using AutoSetup Scheduler, refer to the Application Note "Compensation Setup for High Content DuraClone reagents", downloadable from the Beckman Coulter website: www.duraclone.com/im/.
7. For sample acquisition on other flow cytometers, please follow standard procedures and instrument manufacturer instructions for compensation setup.

## SAMPLE ANALYSIS (Example)

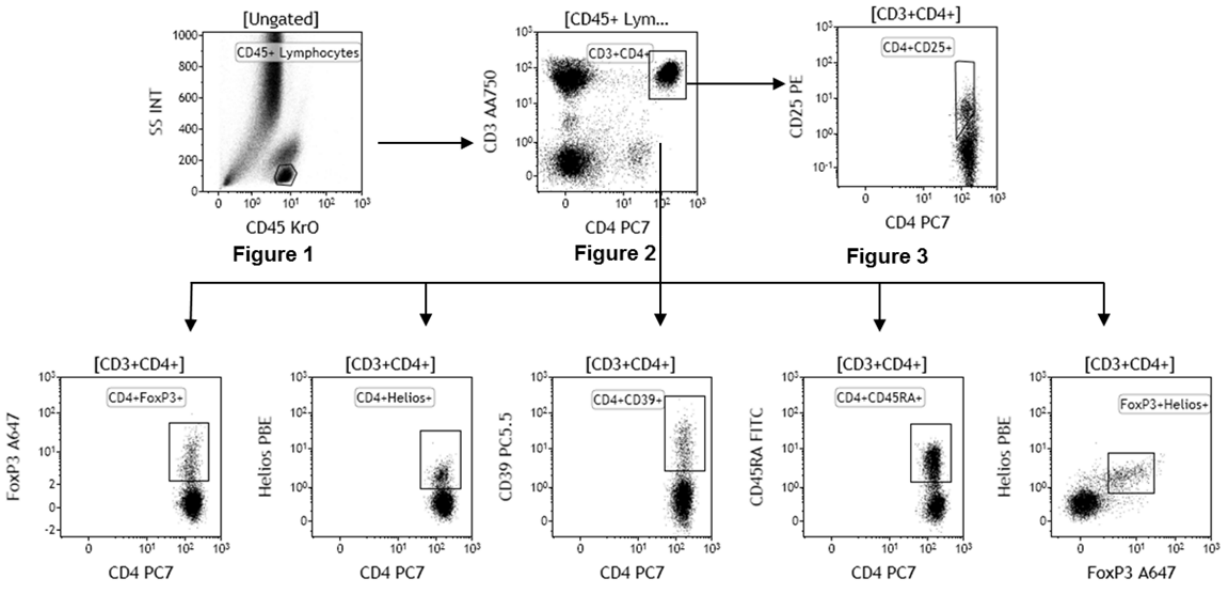


Figure 1 Figure 2 Figure 3 Figure 4 Figure 5 Figure 6 Figure 7 Figure 8

1. Create an appropriate analysis protocol to define the population gates and a series of dual parameter plots for analysis.
2. Set the discriminator on the FS parameter to a value low enough to assure lymphocytes are not excluded from acquisition.
3. Create a CD45-KRO vs. SSC dot plot and apply the leukocyte gate. Create a region to encompass the CD45+ lymphocytes (Figure 1).
4. Create a CD4-PC7 vs. CD3-AA750 dot plot and draw a region to gate the CD3+CD4+ T cells (Figure 2).
5. Create the following dot plots and apply the
6. CD3+CD4+ gate to these plots:
   a. Create a CD4-PC7 vs. CD25-PE plot and gate the CD4+CD25+ population (Figure 3).
   b. Create a CD4-PC7 vs. FoxP3-A647 dot plot and gate the CD4+FoxP3+ population (Figure 4).
   c. Create a CD4-PC7 vs. Helios-PBE plot and gate the CD4+ Helios+ population (Figure 5).
   d. Create a CD4-PC7 vs CD39-PC5.5 plot and gate the CD4+ CD39+ population (Figure 6).
   e. Create a CD4-PC7 vs. CD45RA-FITC plot and gate the CD4+CD45RA+ population Figure 7).
   f. Create a FoxP3-A647 vs. Helios-PBE plot and gate the FoxP3+Helios+ population (Figure 8).

## REFERENCES


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2. Advances in distinguishing natural from induced Foxp3+ regulatory T cells. Xiaohong Lin, Maogen Chen, Ya Liu, Zhiyong Guo, Xiaoshun He, David Brand, Song Guo Zheng. Int J Clin Exp Pathol. 2013.
3. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. Silvia Deaglio, Karen M. Dwyer, Wenda Gao, David Friedman ,Anny Usheva, Anna Erat, Jiang-Fan Chen, Keiichii Enjyoji, Joel Linden, Mohamed Oukka, Vijay K.

Kuchroo, Terry B. Strom and Simon C. Robson. J Exp Med, Jun 11 2007.
4. A peripheral circulating compartment of natural naive CD4 Tregs. Valmori, D., A. Merlo, N. E. Souleimanian, C. S. Hesdorffer, M. Ayyoub. J. Clin. Invest. 2005 (115): 1953–1962.

## PRODUCT AVAILABILITY

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## TRADEMARKS



For additional information, or if a damaged product is received, email Beckman Coulter Customer Service at duraclone-support@beckman.com or contact your local Beckman Coulter Representative.

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