DuraClone Tri T-STAT CD3/CD4/CD8 Reagent Kit

REF **B39492 – 50 tests**

IFU-B39492-1.0

CE BECKMAN COULTER

	Specification of constituent 1	Specification of constituent 2	Specification of constituent 3
Specificity	CD3	CD4	CD8
Clone	UCHT1	RPA-T4	LT8
Immunogen	Purified from mouse using affinity chromatography	Purified from mouse using affinity chromatography	Purified using ion exchange chromatography
Ig Chain	IgG1	IgG1	IgG
Species	Human/chimpanzee	Human/chimpanzee	Human
Source	Mouse	Mouse	Mouse
Fluorochrome	PE-Dyomics649	Atto™488	R-Phycoerythrin
λ Excitation	488 nm	488 nm	488 nm
Emission peak	676 nm	523 nm	575 nm

IVD

INTENDED USE

The DuraClone Tri T-STAT CD3/CD4/CD8 reagent is a three color immunofluorescence stain for the identification and enumeration of helper/inducer (CD4+) cytotoxic/suppressor (CD8+) and total T-lymphocytes (CD3+) combined with a precise number of florescent counting beads for absolute CD3+, CD4+, CD8+, T-Cells counts. This reagent is intended for flow cytometry based analysis in lysed human whole blood samples.

SUMMARY AND EXPLANATION

The reagent contains fluorescently labeled antibodies that bind to CD3, CD4 and CD8 antigens found on the surface of circulating leukocytes. The CD3 antigen is a complex of at least six proteins, known collectively as the T-cell receptor (TCR) complex. The antibody used in this reagent binds to the 20 kDa ε chain of this complex.[1] The CD4 antigen is a 59 kDa protein which interacts with class II molecules of the major histocompatibility complex and is the primary receptor for the Human Immunodeficiency Virus (HIV).[2,3] The CD8 antigen is a complex consisting of two disulfide linked subunits. The antibody used in this reagent binds to the 32 kDa α subunit of the complex. CD8 interacts with class I major histocompatibility complex molecules.[2,3]

PRINCIPLES OF TEST

T-lymphocytes selected through gating of the $CD3^+$ The DuraClone Tri T-STAT CD3/CD4/CD8 reagent consists of murine monoclonal antibodies that specifically recognize the human leukocyte surface antigens CD3, CD4, and CD8. Each of the monoclonal antibodies is labeled with a unique fluorochrome.

Specific cell subsets are stained when blood is combined with the reagent and each monoclonal antibody binds to the cell determinant molecules on the cell surface. Types of cell subset are identified when passed through a flow cytometer laser beam.

The DuraClone Tri T-STAT CD3/CD4/CD8 reagent also contains a precise number of fluorescent beads. When the reagent is combined with a known volume of blood the reagent provides for the single platform determination of the absolute cell concentrations of the stained subsets. The volume of sample analyzed can be determined by multiplication of the total sample volume by the fraction of total beads that were detected during the analysis.

REAGENTS

The DuraClone Tri T-STAT CD3/CD4/CD8 reagent kit contains the following:

- 50 ready-to-use dried down flow cytometer compatible tubes (1 tube/test)
- 1 x 25 mL DuraLyse Solution

REAGENT CONTENTS

The DuraClone Tri T-STAT CD3/CD4/CD8 reagent is formulated in stabilizing buffered saline. It contains Atto488™ – labeled anti-CD4 monoclonal antibody, clone RPA-T4; R-Phycoerythrin (PE) – labeled anti-CD8 monoclonal antibody, clone LT8 and PE-Dyomics649 – labeled anti-CD3 monoclonal antibody, clone UCHT1. The monoclonal antibodies used in DuraClone Tri T-STAT CD3/CD4/CD8 reagent were assigned these specificities at the 8th International Workshop on Human Leukocyte Differentiation Antigens.[4] A precise number of fluorescent counting beads are included in the DuraClone Tri T-STAT CD3/CD4/CD8 reagent to allow single-platform determination of absolute CD4+, CD8+, CD3+ T-cell counts. The reagent is provided in dried form and dispensed in flow cytometer compatible sample tubes with each tube containing one ready-to-use test.

STATEMENT OF WARNINGS

1. Do not use reagent beyond the expiration date on the vial label.
2. To avoid reagent degradation, minimize exposure to light.
3. 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.
4. Use Good Laboratory Practices (GLP) when handling reagent.
5. Dispose all material and samples as per local regulations.
6. DuraLyse Solution contains diethylene glycol and formaldehyde. Formaldehyde is a potential carcinogen and causes irritation to the eyes and skin. Ingestion of diethylene glycol can be fatal.

STORAGE AND HANDLING

Store the reagent between 20-30°C in a dry place and protect from direct exposure to light during storage or sample processing.

After removing the desired number of test tubes, use the resealable plastic bag to store the remaining test tubes. Do not use the test tubes beyond the expiration date on the packaging label.

EVIDENCE OF DETERIORATION

In case of packaging deterioration, damaged product, or if data obtained shows performance alteration, please contact your local distributor or you can contact Beckman Coulter at the following email address: duraclone-support@beckman.com

INSTRUMENT REQUIREMENTS

The DuraClone Tri T-STAT CD3/CD4/CD8 reagent is designed to be used on flow cytometers equipped with 488 nm laser, capable of detecting forward scatter and side scatter, and compatible with the emission spectra of the fluorochromes used in the reagent.

The instrument must be calibrated for setting photomultiplier tube voltages, fluorescence compensation, and the checking of instrument sensitivity according to the manufacturer's guidelines.

SPECIMEN COLLECTION

The venous blood sample should be collected in a sterile blood collection tube containing K_3EDTA. 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 25°C and be used within 24 hours of venipuncture.

PROCEDURE

MATERIALS SUPPLIED

DuraClone Tri T-STAT CD3/CD4/CD8 Reagent Kit
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- DuraClone Tri T-STAT CD3/CD4/CD8 Reagent
- DuraLyse Solution

MATERIALS REQUIRED BUT NOT SUPPLIED

Blood collection tube containing K_3 EDTA
Calibrated pipettes with disposable tips
Vortex mixer
Sheath fluid
Flow Cytometer calibration beads

Assay Protocol

NOTE: At all steps, keep the sample preparations protected from light. Once the sample is lysed, it can be stored for up to 48 hours between 20 - 25°C before running on the flow cytometer.

1. Ensure that the blood sample is adequately mixed. Pipette 50 µL of blood sample into the tube containing the dry down reagent.
2. Vortex the tube to ensure mixing of the blood with the reagent. Incubate for 30 - 40 minutes at room temperature. Protect the tube from exposure to direct light.
3. Add 450 µL of 1X DuraLyse Solution to each tube, and vortex briefly to ensure mixing of sample.
4. Incubate the tubes in the dark for 15 - 20 minutes.
5. Vortex sample tube thoroughly at low speed, and then run on your flow cytometer for acquisition.

Flow Cytometer Acquisition and Analysis

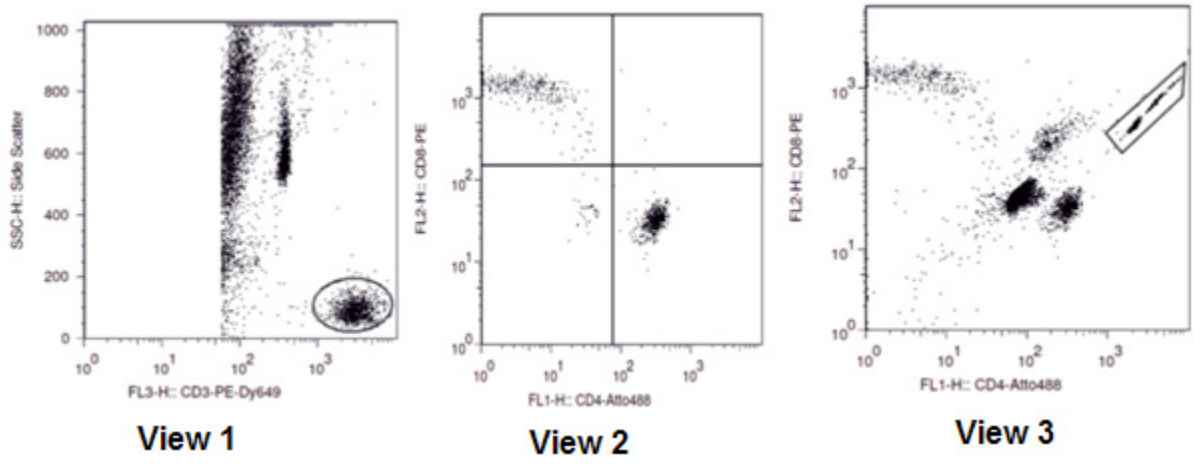
1. Start the flow cytometer according to manufacturer's instructions and calibrate the instrument in accordance with the recommended protocol.

a. For the BD FACSCalibur™ or BD FACScan™ flow cytometers, the instrument should be setup and calibrated using CaliBRITE™ beads.

2. The compensation must be adjusted prior to running the prepared samples.
   a. For the BD FACSCalibur or the BD FACScan, the CaliBRITE settings should be used.
3. Draw three dot-plots (View 1 to View 3).
   a. **View 1**: Anti-CD3 – PE-Dyomics649 (FL-3) vs. Side scatter (Linear scale) – To gate the CD3+ population.
   b. **View 2:** Anti-CD4 - Atto488 (FL-1) vs. Anti-CD8 - PE (FL-2) – Apply Gate CD3+ to capture CD3+CD4+ and CD3+CD8+ events.
   c. **View 3:** Anti-CD4 - Atto488 vs. Anti-CD8 - PE – Un-gated; to gate the bead population.
4. Set the threshold on FL-3 (PE-Dyomics 649) channel to minimize debris.
5. Set 3,000 events on the bead gate as an acquisition stop criteria.
6. The absolute count for each cell type can be calculated using the following equation (where the Gated Cell Count and Bead Count are obtained from the flow cytometer analysis, and the Bead Count per test from the test kit provided):

$$\text{Absolute Cell Count / µL} = \frac{(\text{Gated Cell Count}) \times (\text{Bead Count per test})}{(\text{Gated Bead Count}) \times (\text{Test Volume in µL})}$$

Dot plot views:
View 1: Anti-CD3 – PE-Dyomics649 (FL-3) vs. Side scatter (Linear scale)
View 2: Anti-CD4 - Atto488 (FL-1) vs. Anti-CD8 - PE (FL-2)
View 3: Anti-CD4 - Atto488 vs. Anti-CD8 - PE – Un-gated



PERFORMANCE CHARACTERISTICS

Correlation
The absolute counts for CD3+CD4+ and CD3+CD8+ populations were determined on 96 blood samples using DuraClone Tri T-STAT CD3/CD4/CD8 reagent. These were compared to CYTO-STAT tetraCHROME reagents and BD TriTest™ reagents. The Deming linear regression analysis reported in Table 1 indicates substantial equivalence.

Linearity
Assay linearity was determined by testing six point dilution series, with each level tested in triplicates. The assay was determined to be linear for CD3+CD4+ (64 - 1680 cells/µL) and CD3+CD8+ (32-1640 cells/µL), with correlation (R^2) of 0.995 and 0.999, respectively.

Specificity
The CD3, CD4 and CD8 monoclonal antibodies used in the reagent are assigned to the CD3, CD4 and CD8 cluster of differentiation respectively by the 1st international workshop on Human Leukocyte Differentiation Antigen (HLDA) in Paris, France in 1982.[4] The UCHT1 monoclonal antibody clone of CD3 specifically reacts with the ε chain of the CD3 complex.[5] The RPA-T4 clone binds to the D1 domain (CDR1 and CDR3 epitopes) of the CD4 antigen and reacts with approximately 80% of thymocytes and 45% of peripheral blood lymphocytes.[1]

Reproducibility
This study was performed on a single lot of the reagent, the same day, using same BD FACSCan flow cytometer. A single blood sample was stained by three operators in triplicate following the assay protocol within 24 hours of venipuncture. The results obtained showed similar counts for CD3+, CD4+ and CD8+ populations across the three operators with the coefficient of variance between operators less than 2%. These results are summarized in Table 2.

Table 1. Correlation

	Deming Linear Regression			95% CI	
Parameter	**Slope**	**Y-intercept**	**X-intercept**	**Slope**	**Y-intercept**
Site 1					
CD3+CD4+ T-cell Regression	0.868	-3.28	3.78	0.841 – 0.896	-16.5 – 10.0
CD3+CD8+ T-cell Regression	0.899	35.0	-38.9	0.845 – 0.954	-30.7 – 100
Site 2					
CD3+CD4+ T-cell Regression	0.970	-8.47	8.73	0.917 – 1.02	-34.7 – 17.7
CD3+CD8+ T-cell Regression	1.044	-58.3	55.8	0.978 – 1.11	-136 – 19.3
Site 3					
CD3+CD4+ T-cell Regression	0.982	5.85	-5.95	0.922 – 1.04	-20.0 – 31.7
Site 4					
CD3+CD4+ T-cell Regression	0.901	12.99	-14.42	0.871 – 0.931	-2.67 – 28.7
CD3+CD8+ T-cell Regression	0.884	2.49	-2.82	0.831 – 0.936	-64.3 – 69.3

Table 2. Reproducibility

Combined	CD3 Average	CD4 Average	CD8 Average
Operator 1	1080	394	634
Operator 2	1090	390	637
Operator 3	1116	405	645
Average	1095	396	638
Stdev	18.2	7.9	5.6
%CV	1.66	1.99	0.87

LIMITATIONS

1. The DuraClone Tri T-STAT CD3/CD4/CD8 reagent has only been validated with K_3EDTA treated whole blood.
2. The DuraClone Tri T-STAT CD3/CD4/CD8 reagent is photosensitive and exposure to light may lead to reagent degradation, which in turn would lead to erroneous results.

REFERENCES

1. Knapp W, Dorken B, Gilks WR, Rieber EP, Schmidt RE, Stein H, & von dem BorneAEGKr, eds. Leukocyte typing IV. White cell differentiation antigens. Oxford university press 1989
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4. Bernard, Alain, Laurence Boumsell, and Catherine Hill. Joint report of the first international workshop on human leucocyte differentiation antigens by the investigators of the participating laboratories. Leucocyte Typing. New York, NY: Springer-Verlag 1984: 9-108.
5. Tunnacliffe, Alan, Carina Olsson, and Antonio de la Hera. The majority of human CD3 epitopes are conferred by the epsilon chain. International Immunology 1.5 (1989): 546-550.

PRODUCT AVAILABILITY

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TRADEMARKS

For additional information, or if 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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Printed in India
Made in India

Revision History

Revision 1.0, 09/2013

- Initial Release