DuraClone IF T Activation Tube, 25 Tests, RUO

REF B88649 – 25 tests

IFU- B88649-1.0

	Specifications of Constituent 1	Specifications of Constituent 2	Specifications of Constituent 3	Specifications of Constituent 4	Specifications of Constituent 5	Specifications of Constituent 6
Specificity	IFNγ	TNFα	IL2	CD8	CD3	CD4
Clone	45.15	IPM2	MQ1-17H12	B9.11	UCHT1	13B8.2
Immunogen	Recombinant Human IFN-γ	U266 cell line	E. coli - expressed recombinant human IL2	Human cytotoxic T lymphocyte clone (HLA A2)	T cell line + IL2	Human thymocytes
Isotype	IgG1	IgG1	IgG2a, kappa	IgG1, kappa	IgG1 kappa	IgG1
Species	Mouse	Mouse	Rat	Mouse	Mouse	Mouse
Source	Ascites fluid or supernatant of in vitro cultured hybridoma cells.	Ascites fluid or supernatant of in vitro cultured hybridoma cells.	Purified	Ascites fluid or supernatant of in vitro cultured hybridoma cells	Ascites fluid	Ascites fluid or supernatant of in vitro cultured hybridoma cells.
Purification	Ion exchange or affinity chromatography	Affinity chromatography	Affinity chromatography	Affinity chromatography	Ion exchange or affinity chromatography	Affinity chromatography
Fluorochrome	Fluorescein isothiocyanate (FITC)	R Phycoerythrin (PE)	R Phycoerythrin-Cyanine 7 (PC7)	Alexa Fluor 700 (AF700)	Alexa Fluor 750 (AF750)	Pacific Blue (PBE)
λ Excitation	488 nm	488 nm	488 nm	695 nm	633/638 nm	405 nm
Emission peak	525 nm	575 nm	770 nm	720 nm	783 nm	455 nm

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

BACKGROUND

T cells represent a central subset of the adaptive immune system. Upon recognition of specific antigens T cells - among other responses – secrete inflammatory cytokines such as IFN-γ, TNF-α and IL-2 which stimulate complementary parts of the immune system to defend the host against the provoking pathogens[1]. Identification of cytokine-secreting T cells subsets is an important tool in studying and understanding T cell function in immune activation and suppression.

APPLICATION

The DuraClone IF T Activation Tube, 25 Tests, RUO can be used to identify IFNγ, TNFα and IL2 secreting CD4 and CD8 T cells.

This reagent is intended to be used on a flow cytometer with the features described below:

- A 488 nm laser with detectors dedicated to detection of light scatter (forward and side) and fluorescence emission in the following ranges: 504 – 545 nm, 560 – 600 nm and >755 nm.
- A 638 nm laser with detectors dedicated to detection of fluorescence emission in the following ranges: 715 – 735 nm and >755 nm.
- A 405 nm laser with detectors dedicated to detection of fluorescence emission in the following ranges: 430 – 470 nm.

PRINCIPLE

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by T lymphocyte subpopulations secreting specific cytokines. Specific surface and intracellular staining of the activated T lymphocytes is performed by incubating the sample with DuraClone IF T Activation Tube.

KIT BOX CONTENTS

DuraClone IF T Activation Tube, 25 Tests, RUO contains the following:

- 25 tests of the DuraClone IF T Activation Tube
- 3 Compensation Kits, each kit containing six tubes, each of a single color:
  - CD4-FITC
  - CD4-PE
  - IL2-PC7
  - CD8-AF700
  - CD3-AF750
  - CD4-PBE

STATEMENT OF WARNINGS

1. For stability information of DuraClone IF T Activation Tube, 25 Tests, RUO refer to the Certificate of Analysis (COA).
2. Discard reagent or compensation tubes, as per applicable regulations.
3. Do not store the reagent or compensation tubes in the refrigerator; do not freeze/thaw the tubes.
4. 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.
5. Discard reagent and compensation tubes containing processed samples, as per applicable regulations, after sample acquisition and analysis.
6. 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.
7. Only calibrated instruments, as per the manufacturer's instructions, should be used.
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 protect it 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 such as Navios*, capable of detecting forward and side scatter, and compatible with the emission spectra of the fluorochromes used in the reagent.

SPECIMEN COLLECTION

The whole blood sample should be collected in a blood collection tube containing sodium heparin. Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected. The sample must be stored between 18°C and 25°C. *For other anticoagulants, it is recommended that the user verifies the reagent performance for their specific applications.*

MATERIAL REQUIRED BUT NOT SUPPLIED

- Blood collection tube containing sodium heparin
- Calibrated pipettes
- Vortex mixer
- Sheath fluid
- Flow-Check Pro Fluorospheres (REF. A63493) (For Navios alignment verification)
- Flow-Set Pro Fluorospheres (REF. A63492) (For Navios standardization)
- VersaComp Antibody Capture Beads Kit [Positive Bead] (REF B22804)
- Perfix NC kit (REF. B31168)
- Phosphate Buffered Saline (PBS) (1X)
- Fetal Bovine serum
- Flow cytometer
- Distilled water

NOTE: The sample preparation procedure and compensation setup mentioned in the sections below are for reference purposes only. It may be necessary for users to adapt the protocol as per their specific applications.

PROCEDURE

SAMPLE PREPARATION FOR WHOLE BLOOD (Example)

1. Pipette 50µL of activated blood to an appropriately labeled test tube.(Normal blood contains ~3-11.7 x 10^3 white blood cells per µL)[2]
2. Add 25 µL of buffer R1 (Perfix-nc fixative reagent). Vortex until red pellet is dissociated. Incubate 15 minutes at room temperature.
3. Add 2 mL 1X PBS. Vortex and centrifuge at 150 x g for 5 minutes. Aspirate the supernatant.
4. Add 25 µL of Fetal Bovine Serum. Vortex to re-suspend the pellet, until red pellet is dissociated.
5. Add 300 µL of buffer R2 (Perfix-nc permeabilizing reagent). Vortex.
6. Pipette the entire contents into the DuraClone IF T Activation panel tube. Vortex the tubes at high speed for 6-8 seconds.
7. Incubate 45 minutes at room temperature.
8. Add 3 mL of R3 (final solution 1X in water) (Perfix-nc wash reagent)
9. Vortex and centrifuge 500 x g for 5 minutes. Aspirate the supernatant.
10. Add 500 µL of R3 (final solution 1X in water). Vortex.
11. Sample is ready for acquisition. Analyze the samples immediately on the flow cytometer.

SAMPLE PREPARATION FOR PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) (Example)

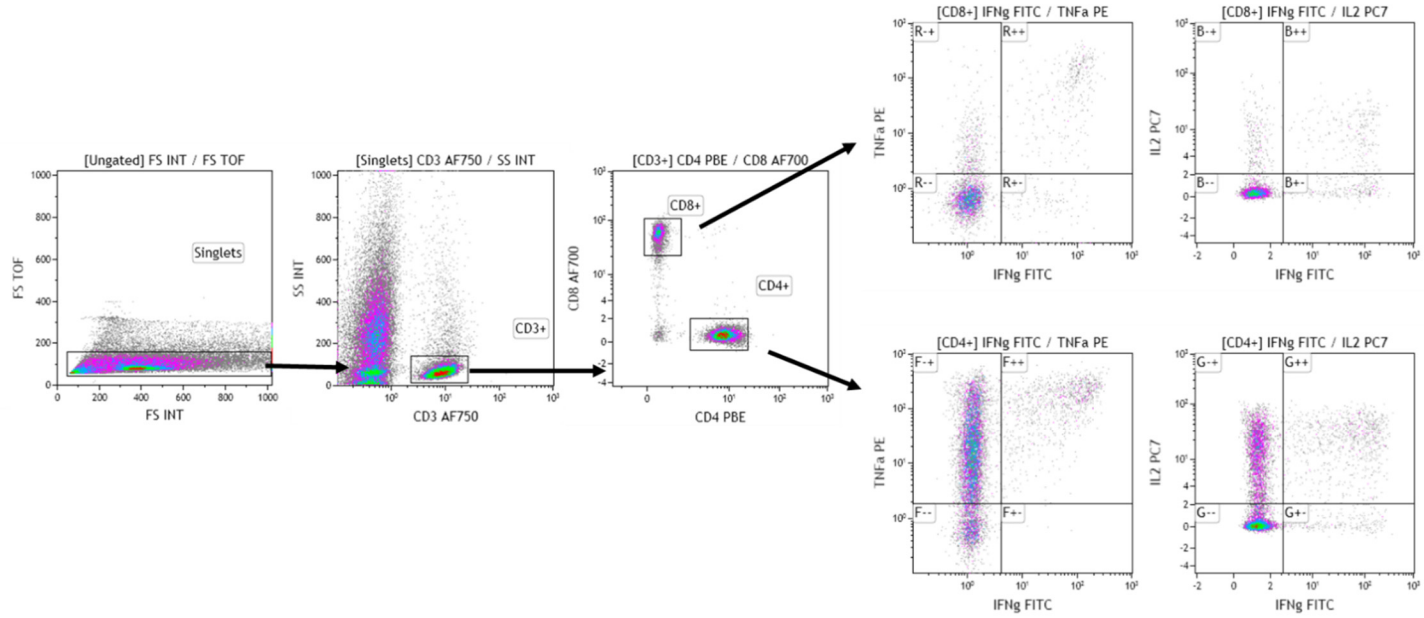
1. Pipette 50 µL of activated PBMCs (containing ~3 x10^5 cells) to an appropriately labeled test tube.
2. Add 25 µL of buffer R1 (Perfix-nc fixative reagent). Vortex until pellet is dissociated. Incubate 15 minutes at room temperature.
3. Add 2 mL 1X PBS. Vortex and centrifuge at 150 x g for 5 minutes. Aspirate the supernatant.
4. Add 25 µL of Fetal Bovine Serum. Vortex to re-suspend the pellet, until pellet is dissociated.
5. Add 300 µL of buffer R2 (Perfix-nc permeabilizing reagent). Vortex.

6. Pipette entire contents into the DuraClone IF T Activation panel tube. Vortex the tubes at high speed for 6-8 seconds.
7. Incubate 45 minutes at room temperature.
8. Add 3 mL of R3 (final solution 1X in water) (Perfix-nc wash reagent).
9. Vortex and centrifuge 500 x g for 5 minutes. Aspirate the supernatant.
10. Add 500 µL of R3 (final solution 1X in water). Vortex.
11. Sample is ready for acquisition. Analyze the samples immediately on the flow cytometer.

COMPENSATION SETUP (Example)

1. Pipette 50 µL of fresh whole blood to each of the six color compensation tubes from a single Compensation Kit provided in the DuraClone IF T Activation Tube, 25 Tests, RUO box.
2. Add one drop of the positive VersaComp antibody capture beads to the following compensation tubes:
   - IL2-PC7 compensation tube
3. Vortex the six compensation tubes at high speed for 6-8 seconds and incubate the tube for 15 minutes, protected from the direct light exposure at 18 to 25ºC.
4. Process the blood samples in all six compensation kit tubes by following steps 2-5 in the Sample Preparation procedure.
5. Do not follow step 6 in the Sample Preparation procedure and proceed with step 7 to 11 as listed in the Sample Preparation procedure.
6. For sample acquisition on Navios/Gallios:
   a. 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: http://beckman.com/applications/immune-monitoring .
7. Ensure that the compensation tubes are run in the following order:
   - CD4-FITC
   - CD4-PE
   - IL2-PC7
   - CD8-AF700
   - CD3-AF750
   - CD4-PBE
8. For sample acquisition on other flow cytometers, please follow standard procedures and instrument manufacturer instructions for application and compensation setup.

SAMPLE ANALYSIS (Figure 1: Example)



1. Create an appropriate analysis protocol to define the population gates and a series of dual parameter plots for analysis as shown above. (Figure 1)
2. Set the discriminator on the FS parameter to a value low enough to assure lymphocytes are not excluded from acquisition.
3. Create a FS INT vs FS TOF dot plot and a singlet gate to eliminate any doublets (identified by higher values on FS TOF)
4. Create a CD3-AF750 vs. SSC dot plot and apply the singlets gate. Create a region to encompass the CD3+ cells.
5. Create a CD4-PBE vs. CD8 AF700 dot plot and apply the CD3+ gate. Create two regions to encompass the CD4+ and CD8+ cells individually.
6. On the CD4+ and CD8+ gated cells assess the cytokine production by:
   a. Creating an IFNγ FITC vs TNFα PE dot plot and a quadrant gate to differentiate subsets: IFNγ+ TNFα-, IFNγ+ TNFα+, IFNγ- TNFα+ and IFNγ- TNFα- cells.
   b. Creating an IFNγ FITC vs IL2 PC7 dot plot and a quadrant gate to differentiate subsets IFNγ+ IL2-, IFNγ+ IL2+, IFNγ- IL2+ and IFNγ- IL2- cells.

REFERENCES

1. OMIP-025: evaluation of human T- and NK-cell responses including memory and follicular helper phenotype by intracellular cytokine staining. Moncunill G, Dobaño C, McElrath MJ, De Rosa SC. Cytometry A. 2015 Apr;87(4):289-92.
2. Hematological and iron-related analytes—Reference data for persons aged 1 year and over: United States, 1988–94. Hollowell JG, Van Assendelft OW, Gunter EW, Lewis BG, Najjar M, Pfeiffer C. National Center for Health Statistics. Vital Health Stat 11(247),2005.

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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