DuraClone IF T Helper Cell Tube, 25 Tests, RUO

REF C04666 – 25 tests

IFU- C04666-1.0

	Specifications of Constituent 1	Specifications of Constituent 2	Specifications of Constituent 3	Specifications of Constituent 4	Specifications of Constituent 5
Specificity	IFNγ	IL-4	CD4	CD3	IL-17a
Clone	45.15	MP4-25D2	13B8.2	UCHT1	BL168
Immunogen	Recombinant Human IFN-γ	CHO-expressed, recombinant human IL-4	Human thymocytes	T cell line + IL2	Recombinant full length human IL-17A
Isotype	IgG1	IgG1	IgG1	IgG1 kappa	IgG1 kappa
Species	Mouse	Rat	Mouse	Mouse	Mouse
Source	Ascites fluid or supernatant of in vitro cultured hybridoma cells.	Purified	Ascites fluid or supernatant of in vitro cultured hybridoma cells.	Ascites fluid	Purified
Purification	Ion exchange or affinity chromatography	Affinity chromatography	Ion exchange or affinity chromatography	Ion exchange or affinity chromatography	Affinity chromatography
Fluorochrome	Fluorescein isothiocyanate (FITC)	R Phycoerythrin-Cyanine 7 (PC7)	Allophycocyanin (APC)	Alexa Fluor 750 (AF750)	Pacific Blue (PB)
λ Excitation	488 nm	488 nm	633/638nm	633/638 nm	405 nm
Emission peak	525 nm	770 nm	660 nm	783 nm	455 nm

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

BACKGROUND

CD4+ T cells represent the majority of T lymphocytes in the secondary lymphoid organs. They preferentially recognize HLA Class II and their activation leads to their differentiation into subsets depending on the context of the stimuli[1]. Activated CD4+ T cell subsets can be identified by their signature cytokines like IL-4, IL-17a and/or IFN-γ which are important for their helper cell functions.

APPLICATION

The DuraClone IF T Helper Cell Tube, 25 Tests, RUO can be used to identify IFNγ-, IL-4- and IL-17a-secreting CD4+ 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, and >755 nm.
- A 638 nm laser with detectors dedicated to detection of fluorescence emission in the following ranges: 650-670 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 Helper Cell Tube.

KIT BOX CONTENTS

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

- 25 tests of the DuraClone IF T Helper Cell Tube
- 3 Compensation Kits, each kit containing five tubes, each of a single color:
  - CD4-FITC
  - IL4-PC7
  - CD4-APC
  - CD3-AF750
  - CD4-PB

STATEMENT OF WARNINGS

1. For stability information of DuraClone IF T Helper Cell 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 (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 50 µL 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 200 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 Helper Cell 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)

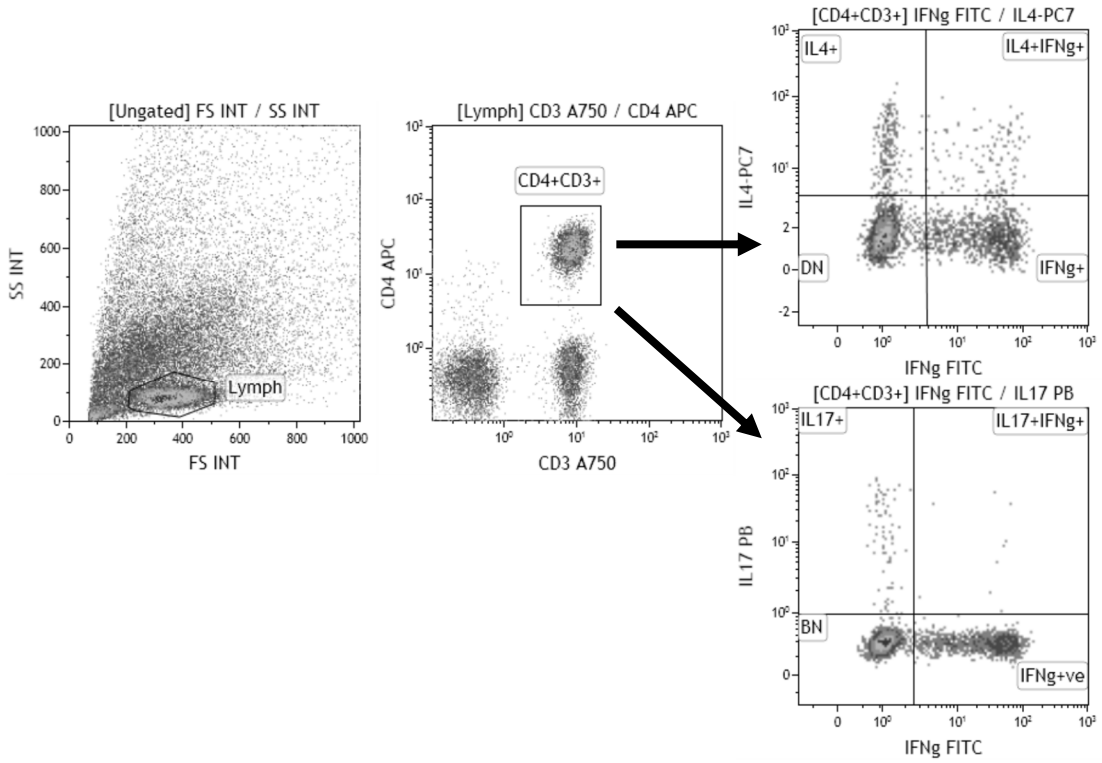
1. Pipette 50 µL of activated PBMCs (containing ~5 x10^5 cells) to an appropriately labeled test tube.
2. Add 25 µL of buffer R1 (Perfix-nc fixative reagent). Vortex until pellet is dissociated. Incubate 15 minutes at room temperature.
3. Add 2 mL 1X PBS. Vortex and centrifuge at 200 x g for 5 minutes. 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 Helper Cell 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 five color compensation tubes from a single Compensation Kit provided in the DuraClone IF T Helper Cell Tube, 25 Tests, RUO box.
2. Add one drop of the positive VersaComp antibody capture beads to the following compensation tubes:
   - IL4-PC7 compensation tube
3. Vortex the five 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 five compensation single tubes by following steps 2-5 in the Sample Preparation procedure for whole blood.
5. Proceed with step 7 to 11 (exclude step 6) as listed in the Sample Preparation procedure (for whole blood).
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
   - IL4-PC7
   - CD4-APC
   - CD3-AF750
   - CD4-PB
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 CD3-AF750 vs. CD4-APC dot plot. Create a region to encompass the CD3+CD4+ cells.
4. On the CD4+CD3+ gated cells assess the cytokine production by:
   a. Creating an IFNγ FITC vs IL4 PC7 dot plot and a quadrant gate to differentiate subsets: IFNγ+ IL4-, IFNγ+ IL4+, IFNγ- IL4+ and IFNγ- IL4- cells.
   b. Creating an IFNγ FITC vs IL17a PBE dot plot and a quadrant gate to differentiate subsets IFNγ+ IL17a-, IFNγ+ IL17a+, IFNγ- IL17a+ and IFNγ- IL17a- cells.

REFERENCES

1. CD4+T Cells: Differentiation and Functions. Rishi Vishal Luckheeram, Rui Zhou, Asha Devi Verma, and Bing Xia. Clinical and Developmental Immunology. 2012.
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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REF C04666

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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