SARTORIUS

Product Guide

iQue® T Cell Phenotyping Kit
(CD3, CD4 and CD8)

Product Information

Presentation, Storage and Stability

The iQue® T Cell Kit Phenotyping Kit contains reagents for measurement of T cell phenotyping.

Compatible with iQue® platform with VBR option		
Product Name	**Cat No**	**Format**
iQue® T Cell Kit Phenotyping Kit	97099	1x96 wells
iQue® T Cell Kit Phenotyping Kit	97100	5x96 wells
iQue® T Cell Kit Phenotyping Kit	97101	1x384 wells
iQue® T Cell Kit Phenotyping Kit	97102	5x384wells

Table 1. Product Information
Note: The 1x384-well kit has enough reagents to run 2x96-well plates, NOT 4x96-well plates.

Kit Components	Amount	Storage	Stability
Human T Cell Phenotyping (TCP) Antibody Panel Detection Cocktail	1 vial	2-8°C	Minimum 6 month shelf life; up to one year
Detection Enhancing Solution	1 vial	2-8°C	
Wash Buffer	1 bottle	2-8°C	

Table 2. Kit Components and Storage
Note: A kit manual and a USB key with assay templates are also included in the kit Package.

Background

The iQue® T Cell Phenotyping Kit (CD3, CD4 and CD8) is designed for reliable identification of human T cell subsets. This assay is optimized to run on the iQue® 3 (VBR and VYB configurations) platform combining high throughput sampling, flow cytometry detection and multiplexing capabilities.

The kit is formulated to minimize non-specific background staining. The optimized workflow also provides the flexibility by enabling additional markers and cytokines to be added for further characterization of subpopulations.

Quick Guide

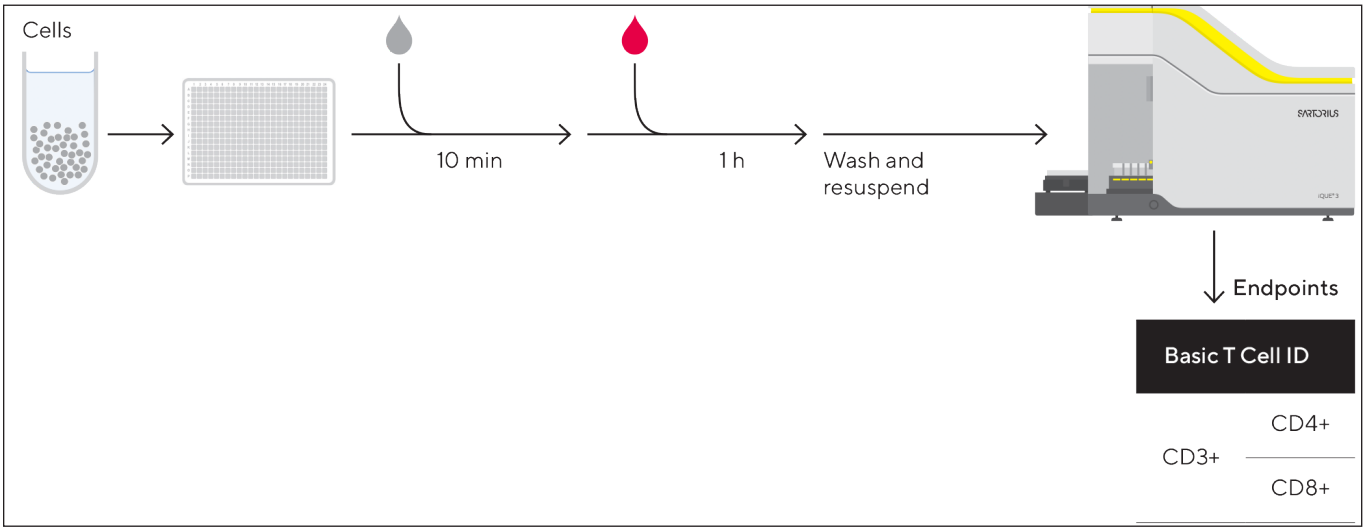

Figure 1: iQue® T Cell Phenotyping Kit workflow. Cell samples are added to the assay plate. After a wash, the cells are incubated with Detection Enhancing Solution followed by addition of T Cell Phenotyping Antibody Panel Detection Cocktail. The cells are then washed, re-suspended with buffer, and analyzed with an iQue® 3 instrument.

Recommended Use

The iQue® T Cell Phenotyping Kit is used to detect T cell surface markers CD3, CD4 and CD8 by flow cytometry. Samples can be cultured cells, immediately thawed frozen cells, and lyophilized cells. Please follow the appropriate protocols to culture, thaw, or reconstitute cells.

Protocols and Procedures for 96 and 384 well plates

Protocols for T Cell Staining

Protocol for 1 x 96-well Plate

1. Add Cell Sample to the assay plate

1.1 Mix cells in the original culture plate or tube by pipetting to break down any cell aggregates. Be careful not to introduce bubbles in the samples.

1.2 Transfer 10 µL of cells to each well of the assay plate designated as Sample during the plate set up on the iQue Forecyt® Design tab.

1.3 Add 190 µL of fresh culture media or cell staining buffer of choice to each assay well.

1.4 Spin the assay plate (300g, 5 minutes).

1.5 Aspirate the supernatants and agitate the samples in the residual liquid in the plate on the shaker feature on the iQue® platform (3,000 rpm, 60 seconds).

2. Add Detection Enhancing Solution

2.1 Add 10 µL/well Detection Enhancing Solution to the assay plate.

2.2 Do a quick spin (300g, 5 seconds) to ensure that all samples are at the well bottom.

2.3 Briefly shake the plate for 20 seconds at 2,000 rpm using the shaker feature on the iQue® platform for thorough mixing.

2.4 Cover the plate to prevent vaporization.

2.5 Incubate the plate at room temperature for 10 minutes.

3. Add Antibody Panel Detection Cocktail

3.1 Add 10 µL/well antibody panel detection cocktail to the assay plate.

3.2 Quick spin (300g, 5 seconds) and brief shake (2,000rpm, 20 seconds) of the plate.

3.3 Cover and incubate the plate at room temperature for 30-60 minutes protecting from light.

4. Wash/Resuspension

4.1 Add 100 µL/well of wash buffer provided in the kit to the assay plate.

4.2 Spin the assay plate (300g, 5 minutes).

4.3 Aspirate the supernatants and agitate the samples in the residual liquid in the plate on the shaker feature on the iQue® platform (3,000 rpm, 60 seconds).

4.4 Add 20 µL/well wash buffer.

4.5 Do a quick spin (300g, 5 seconds).

4.6 The samples are now ready for acquisition on iQue® 3 (VBR or VYB) platform.

Protocol for 1 x 384-well Plate

1. Add Cell Sample to the assay plate

1.1 Mix cells in the original culture plate or tube by pipetting to break down any cell aggregates. Be careful not to introduce bubbles in the samples.

1.2 Transfer 5 µL of cells to each well of the assay plate designated as Sample during the plate set up on the iQue Forecyt® Design tab.

1.3 Add 95 µL of fresh culture media or cell staining buffer of choice to each assay well.

1.4 Spin the assay plate (300g, 5 minutes).

1.5 Aspirate the supernatants and agitate the samples in the residual liquid in the plate on the shaker feature on the iQue® platform (3,000 rpm, 60 seconds).

2. Add Detection Enhancing Solution

2.1 Add 5 µL/well Detection Enhancing Solution to the assay plate.

2.2 Do a quick spin (300g, 5 seconds) to ensure that all samples are at the well bottom.

2.3 Briefly shake the plate for 20 seconds at 2,000 rpm using the shaker feature on the iQue® platform for thorough mixing.

2.4 Cover the plate to prevent vaporization.

2.5 Incubate the plate at room temperature for 10 minutes.

3. Add Antibody Panel Detection Cocktail

3.1 Add 5 µL/well antibody panel detection cocktail to the assay plate.

3.2 Quick spin (300g, 5 seconds) and brief shake (2,000rpm, 20 seconds) of the plate.

3.3 Cover and incubate the plate at room temperature for 30–60 minutes protecting from light.

4. Wash/Resuspension

4.1 Add 50 µL/well of wash buffer provided in the kit to the assay plate.

4.2 Spin the assay plate (300g, 5 minutes).

4.3 Aspirate the supernatants and agitate the samples in the residual liquid in the plate on the iQue® plate shaker (3,000 rpm, 60 seconds).

4.4 Add 10 µL/well wash buffer.

4.5 Do a quick spin (300g, 5 seconds).

4.6 The samples are now ready for acquisition on iQue® 3 (VBR or VYB) platform.

Protocol for Combining T Cell Staining with Qbeads Cytokine Assay

1. Prepare Cytokine Standard

Follow the instructions specified for individual Qbeads Panel to reconstitute the cytokine standard and perform serial dilution using fresh culture media.

2. Prepare Cytokine Capture Beads

2.1 Vortex the Cytokine Capture Beads vials for at least 15 seconds before use.

2.2 In a 15 mL conical tube, combine 40 µL (for one 96-well plate) or 90 µL (for one 384-well plate) of 50 x capture beads for each cytokine.

2.3 Add Human Capture Bead Buffer to bring the total volume to 2 mL (96-well) or 4.5 mL (384-well).

2.4 Further dilute the 1 x Capture Beads with 18-fold volume of fresh media, i.e. 36 mL for the 96-well plate and 81 mL for the 384-well plate.

3. Add Cell/Supernatant Mixture Sample and Cytokine Standard

3.1 Mix the cell/supernatant mixture in the original culture plate by pipetting to break down cell aggregates. Be careful not to introduce bubbles in the samples.

3.2 Transfer 10 µL (96-well) or 5 µL (384-well) of cell/supernatant sample to each well of the assay plate designated as Sample during the plate set up in the iQue Forecyt® Design tab.

3.3 Transfer same volume of cytokine standards to each well of the assay plate designated for Standards in the iQue Forecyt® Design tab.

4. Add the Pre-diluted Cytokine Capture Beads

4.1 Vigorously vortex the pre-diluted Cytokine capture beads prepared earlier.

4.2 Add 190 µL (96-well) or 95 µL (384-well) of the above beads to each assay well. Agitate the beads occasionally to prevent the bead settling.

Note: During the liquid transfer, change the tip to avoid cross-well contamination.

4.3 Cover the plate to prevent evaporation.

4.4 Incubate the plate at room temperature for 60 minutes.

Note: Do NOT shake the plate to prevent the liquid volume in assay wells from splashing into adjoining wells.

4.5 Spin the assay plate (300g, 5 minutes).

4.6 Aspirate the supernatants and agitate the samples in the residual liquid in the plate on the shaker feature on the iQue® platform (3,000 rpm, 60 seconds).

5. Add Cytokine Detection Cocktail

5.1 Add 10 µL/well (96-well) or 5 µL/well (384-well) cytokine detection cocktail to the assay plate.

5.2 Quick spin (300g, 5 seconds) the plate.

5.3 Mix the plate for 20 seconds at 2,000 rpm using the shaker feature on the iQue® platform for thorough mixing.

5.4 Cover the plate to prevent evaporation and protect from light.

5.5 Incubate the plate at room temperature for 60 minutes.

6. Add Antibody Panel Detection Cocktail

6.1 Add 10 µL/well (96-well) or 5 µL/well (384-well) antibody panel detection cocktail to the assay plate.

6.2 Quick spin (300g, 5 seconds) and brief shake (2,000 rpm, 20 seconds) of the plate.

6.3 Cover and incubate the plate at room temperature for 60 minutes protecting from light.

7. Wash/Resuspension

7.1 Add 100 µL/well (96-well) or 50 µL/well (384-well) of wash buffer provided in the kit to the assay plate.

7.2 Spin the assay plate (300g, 5 minutes).

7.3 Aspirate the supernatants and agitate the samples in the residual liquid in the plate on the shaker feature on the iQue® platform (3,000 rpm, 60 seconds).

7.4 Add 20 µL/well (96-well) and 10 µL/well (384-well) wash buffer.

7.5 Do a quick spin (300g, 5 seconds).

7.6 The samples are now ready for acquisition on iQue® 3 (VBR or VYB) platform.

General Guidelines

1. Use a Plate Washer for Aspiration

For wash/aspiration steps, we recommend to use an automated plate washer. Manual aspiration of plates and/or plate inversion techniques could result in severe sample loss. Follow the manufacturer's instruction to set up the plate wash program.

2. Adjust Sip Time to Acquire Enough Cells

2.1 Sip time determines how many cell events are acquired from each well. The template in the kit has a default sip time of 4 seconds per well. Increase it to acquire enough cell events for your data analysis to reach statistical significance of your cell population of interest. If you use a longer sip time than the default 4-second pre-set in the template, you may also need to adjust inter-well shaking in iQue Forecyt® Protocol.

2.2 If you anticipate that you will not have enough cell events even by increasing the sip time, please see section 3 below to increase cell number acquisition before transferring samples to the assay plate.

3. Adjust cell density if necessary.

3.1 Spin cells down (300 g, 5 minutes) in the original cell culture plate or tube.

3.2 Remove half or two thirds the volume of supernatant to double or triple cell density.

3.3 Re-suspend cells in the original culture plate or tube in the remaining supernatant by manually pipetting the sample up and down (5-6 times).

4. (Optional) Include a cell viability dye

We strongly recommend to include a cell viability dye for distinguishing live and dead cells in your assay. Follow the manufacturer's protocol to stain the cells with the viability reagent. Below is an example of using iQue® Cell Membrane Integrity (R/Red) Dye for staining.

4.1 Add 1 µL iQue® Cell Membrane Integrity (R/Red) Dye per 500 µL Antibody Panel Detection Cocktail right before the assay.

4.2 After dye addition, mix the dye by manually pipetting the solution up and down 5 times.

Sample Acquisition and Data Analysis

1. Launch iQue Forecyt® software.
2. Import the provided experiment template (included on USB key in the kit package). Create a New Experiment using the template.
3. In the Design tab, assign wells to samples.
4. In the Protocol tab, adjust the sip time if necessary.
5. Click "RUN" in the Controller to acquire the plate.
6. Use the provided analysis template for gating and data analysis.
   Note: The template gates are pre-set for different populations. Manually draw the gates or fine tune the existing gates from the template if needed.

Example Data

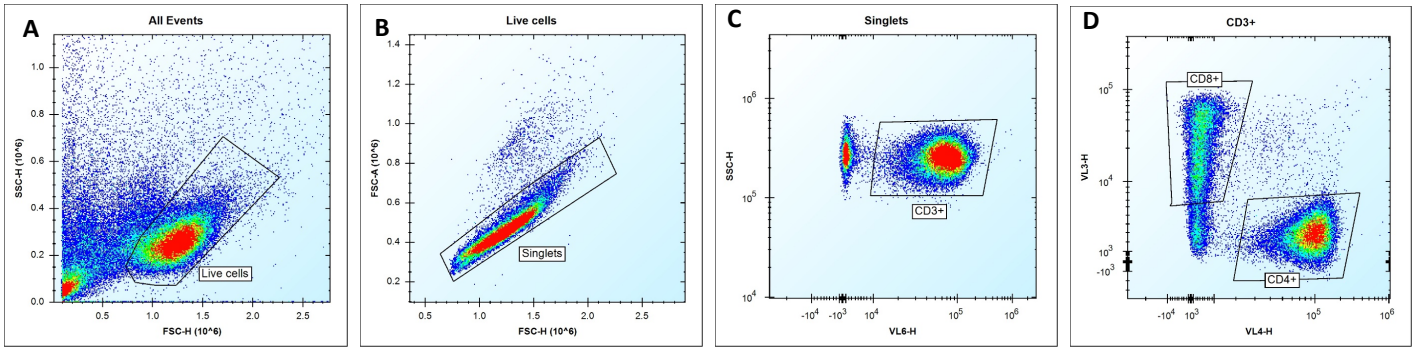

Figure 2: Phenotyping analysis of human PBMCs cultured for one day. A. Set gate for live cells in all events to exclude debris | dead cells. B. Remove doublets from live cells to obtain single cells. C. $CD3^+$ cells of Singlets. D. $CD4^+$ and $CD8^+$ subsets of $CD3^+$ cells.

Sales and Service Contacts

For further information, visit www.sartorius.com

Sartorius BioAnalytical Instruments, Inc.

www.sartorius.com/ique

Publication No. #17199 Rev B.

North America
Sartorius Corporation
300 West Morgan Road
Ann Arbor, Michigan, 48108
Telephone +1 734 769 1600
E-Mail: AskAScientist@sartorius.com
Online Store: shop.intellicyt.com

Europe
Sartorius UK
Units 2 & 3 The Quadrant
Newark Close
Royston Hertfordshire
SG8 5HL
United Kingdom
Telephone +44 (0) 1763 227400
E-Mail: euorders.UK03@sartorius.com

APAC
Sartorius Japan
4th floor Daiwa Shinagawa North Bldg.
1-8-11 Kita-Shinagawa
Shinagawa-ku, Tokyo
140-0001
Japan
Telephone: +81 3 6478 5202
E-Mail: orders.US07@sartorius.com