SARTORIUS

Product Guide

iQue® Human Antibody Dependent Cellular Phagocytosis (ADCP) Kit

Product Information

Presentation, Storage and Stability

The iQue® Human Antibody Dependent Cellular Phagocytosis (ADCP) Kit contains a sufficient quantity of reagents for measurement of ADCP in 1x96, 5x96, 1x384, 5x384-well format. Included in the kit are iQue® Proliferation and Encoding B/Green Dye, iQue® Cell Membrane Integrity R/Red Dye, Human ADCP Antibody Detection Reagent, Wash Buffer and USB Flash Drive Containing Analysis Templates.

Upon receipt, the Human ADCP Antibody Detection Reagent and Wash Buffer should be stored at 2-8°C. The iQue® Proliferation and Encoding B/Green Dye and iQue® Cell Membrane Integrity R/Red Dye should be stored at -20°C. The kit is stable for at least 6 months from the date of receipt.

Product Name	Cat. No.	Format
iQue® Human Antibody Dependent Cellular Phagocytosis (ADCP) Kit	BA-97106	1x96-well
	BA-97107	5x96-well
	BA-97108	1x384-well
	BA-97109	5x384-well

Kit Components	Cat. No. BA-97106 1x96-well	Cat. No. BA-97107 5x96-well	Cat. No. BA-97108 1x384-well	Cat. No. BA-97109 5x384-well	Storage	Stability
iQue® Proliferation and Encoding B/Green Dye	1 vial (25 µL)	5 vials (25 µL)	1 vial (25 µL)	5 vials (25 µL)	-20°C	6 months from date of receipt
iQue® Cell Membrane Integrity R/Red Dye	1 vial (250 µL)	5 vials (250 µL)	1 vial (250 µL)	5 vials (250 µL)	-20°C	
Human ADCP Antibody Detection Reagent	1 bottle (2 mL)	5 bottles (2 mL)	1 bottle (5.4 mL)	5 bottles (5.4 mL)	2-8°C	
Wash Buffer	1 bottle (25 mL)	1 bottle (125 mL)	1 bottle (25 mL)	1 bottle (125 mL)	2-8°C	

Note: A kit manual and a USB key with assay templates are also included the kit package.

Background

The iQue® Human Antibody Dependent Cellular Phagocytosis (ADCP) Kit enables high throughput identification of tumor elimination mediated by mAbs. The kit is designed for homogeneous labeling of target cancer cells using a green fluorescent dye, effector cell identification via (non-adherent or adherent) CD14+ labeling, as well as quantification of cell membrane integrity. Post labeling of target and effector cells, customer test antibodies are added, inducing tumor clearance via their binding to encoded target cells and subsequent CD14+ effector cell binding and engulfment. Test antibodies bind to target cells through its Fab region and CD14+ effector cells binding via Fc region. The readout measures the number of CD14+ effector cells that are positive for the cell encoder dye. The CD14+ effector cells can only be positive for the cell encoder dye if they have phagocytosed target cells. The iQue® Human ADCP Kit has been validated for use on the iQue® platform with BR and VBR configurations.

Recommended Use

Target cells are labeled with iQue® Proliferation and Encoding (B/Green) Dye, added to 96- or 384-well plates, and incubated with test antibody before combining with effector cells. ADCP is measured by first identifying the live cells using iQue® Cell Membrane Integrity (R/Red) Dye, then CD14+ target cells as a percent of live target cells.

This assay has been validated using Raji and Ramos cell lines as targets, anti-CD20 test antibody, and purified monocytes (1:5) or PBMCs (1:20) as effectors.

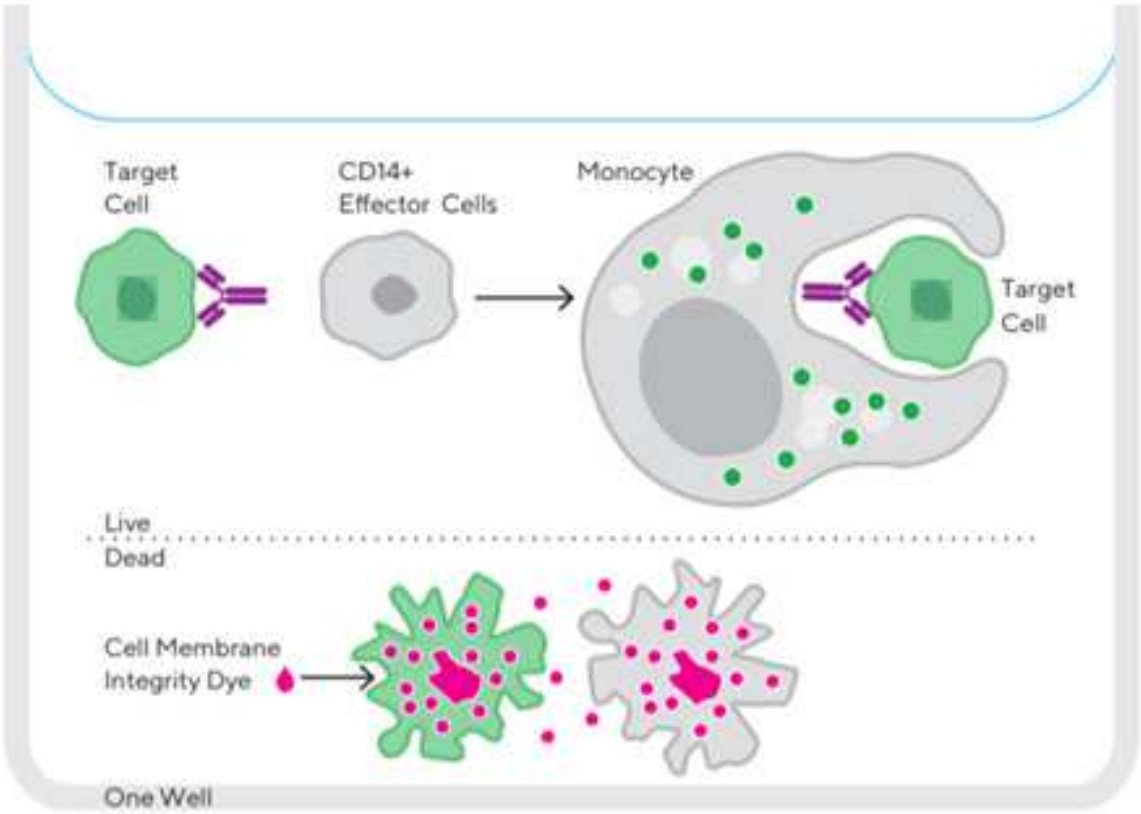

Figure 1: Overview of iQue® Human ADCP Kit.

Workflow

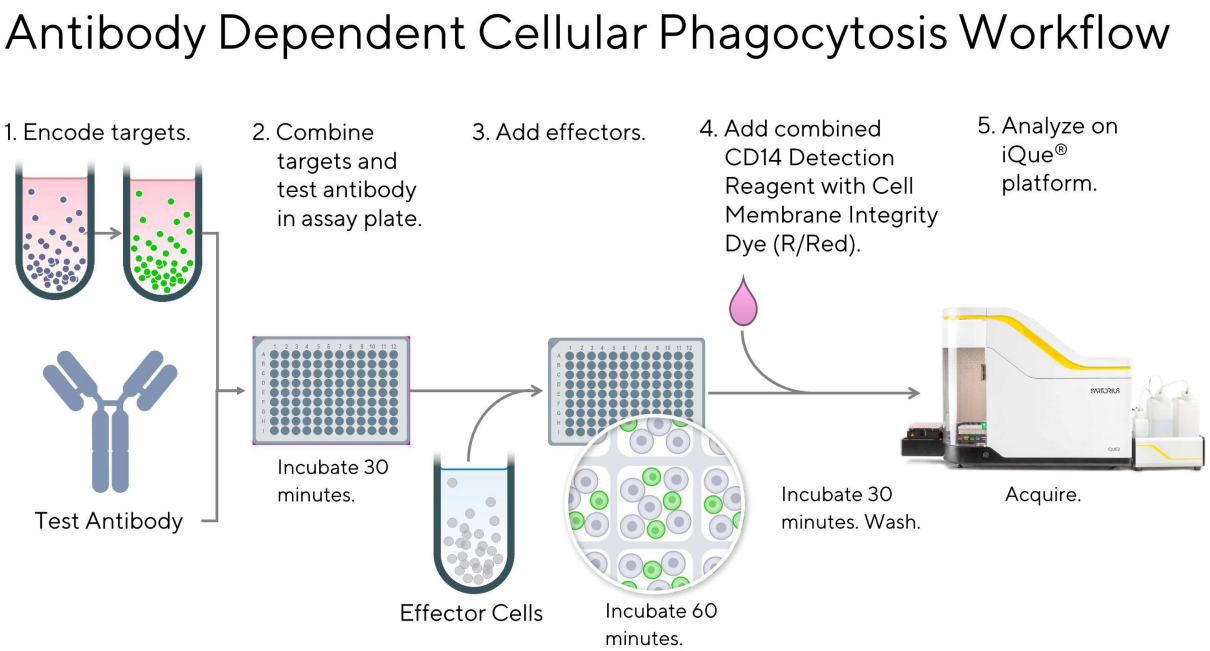

Figure 2: iQue® Human ADCP Kit workflow.

Protocol and Procedure for 96 and 384-well plates

1. Prepare Effector Cells

1.1 Thaw frozen monocytes or PBMCs per supplier instructions.

1.2 Allow cells to rest overnight in a conical tube (NOT a tissue culture coated flask) in an incubator (5% CO_2, 37°C), with a loose cap.

1.3 Before beginning assay, adjust cell density to 2 x 10^6 for PBMCs or 5 x 10^5 for purified monocytes using appropriate culture medium (1:20 or 1:5 target-to-effector ratio, respectively).

Note: Monocytes will stick to tissue culture flasks. Culturing in conical tubes minimizes sticking and prevents monocyte loss.

2. Encode Target Cells

2.1 Before beginning, ensure that the iQue® Proliferation and Encoding (B/Green) Dye is completely thawed.

2.2 Prepare 2X working solution by diluting the iQue® Proliferation and Encoding (B/Green) Dye into a protein-free buffer such as Hank's Balanced Salt Solution (HBSS) or Phosphate Buffered Saline (PBS) at a dilution factor of 1:1250.

2.3 Spin cells down in a conical tube (500 x g, 5 minutes) and remove the original culture medium.

2.4 Resuspend cells in 20 mL protein-free HBSS or PBS. Spin cells down (500 x g, 5 minutes). Remove the supernatant. Resuspend cells in the same buffer at 2 x 10^6 cells/mL.

2.5 Combine an equal volume of cells and the prepared 2X dye working solution. Thoroughly mix, and incubate cells at room temperature for 15 minutes, protect from light.

2.6 After staining, wash by adding at least 2X volume of complete culture medium (with 10% serum) to the staining sample. Spin (500 x g, 5 minutes). Remove the supernatant.

2.7 Repeat wash two more times.

2.8 After final wash, carefully resuspend cells at desired cell density for assay (1 x 10^5 cells/mL), and place conical tube in incubator (5% CO_2, 37°C) with loose cap until use.

3. Prepare Test Antibody Dilutions

Prepare a dilution series of test antibody (customer provided) using culture medium. Ensure enough volume for 25 µL of test antibody per well.

4. Prepare Human ADCP Antibody Detection Cocktail

4.1 Before beginning, ensure that the iQue® Cell Membrane Integrity (R/Red) Dye is completely thawed.

4.2 Dilute the iQue® Cell Membrane Integrity (R/Red) Dye 1:60 in Human ADCP Antibody Detection Reagent. The kit provides enough volume for 10 µL per well with overage for the specified kit size (96- or 384-well).

4.3 Store the ADCP Antibody Detection Cocktail at 4°C until needed.

Note: During liquid transfers, change pipette tips to avoid cross-well contamination

5. Perform Assay

5.1 Transfer **25 µL of test antibody** to each well of the assay plate. We recommend including a "zero" (medium alone) and plating samples from low to high (see Figure 3).

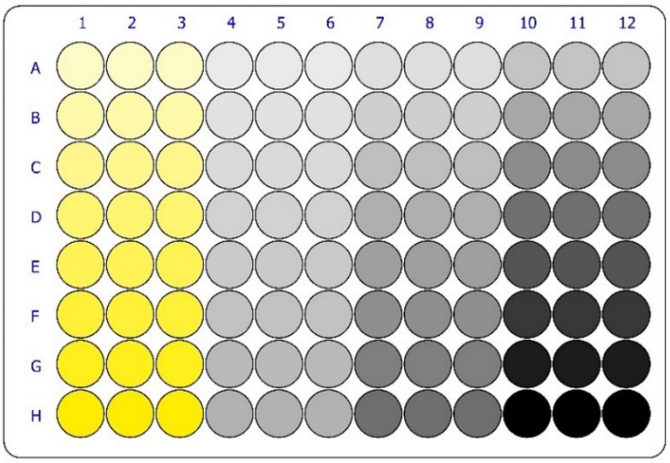

Figure 3: Recommended plating order.

5.2 Transfer **25 µL of encoded target cells** to each well of the assay plate containing test antibody. Give the assay plate a quick spin (300 x g, 5 seconds) to ensure that all samples are at the well bottom.

5.3 Incubate plate for 30 minutes at room temperature, protect from light.

5.4 Transfer **25 µL of effector cells** to each well of the assay plate. Give the assay plate a quick spin (300 x g, 5 seconds) to ensure that all samples are at the well bottom.

5.5 Incubate plate for 1 hour at room temperature, protect from light.

5.6 Transfer **10 µL of cold ADCP Antibody Detection Cocktail** to each well of the assay plate. Give the assay plate a quick spin (300 x g, 5 seconds) to ensure that all samples are at the well bottom.

5.7 Incubate plate for 30 minutes at room temperature, protect from light.

5.8 After incubation, spin the assay plate (300 x g, 5 minutes).

5.9 Aspirate the supernatant.

5.10 Shake the plate (3000 RPM, 60 seconds) to resuspend cells in residual liquid.

5.11 Add **20 µL cold Wash Buffer** to each well.

5.12 Give the assay plate a quick spin (300 x g, 5 seconds) to ensure that all samples are at the well bottom.

6. Plate Acquisition and Data Analysis

6.1 Launch iQue Forecyt® Software.

6.2 Import the provided experiment template (included on USB key in the kit package).

6.3 Create a New Experiment using the provided template.

6.4 In the Design section, assign wells to Sample. In the Series subsection, edit/add Series to ensure proper plate layout.

6.5 In the Protocol section, adjust Sample Order if plate layout requires (horizontal instead of vertical). Acquire samples from low concentration to high to minimize carryover.

6.6 Click "Run" on the Controller window to acquire the plate.

6.7 Use template to gate Cells → Live Cells → Encoded → CD14+ (see Figure 4).

6.8 Percent ADCP is calculated from CD14+ population as a percent of Encoded (see Figure 5).

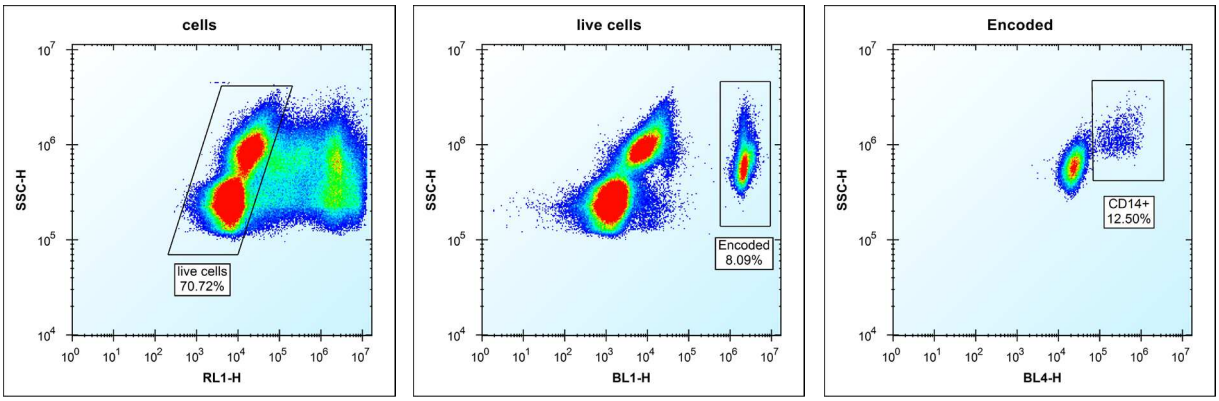

Figure 4: iQue® Human ADCP Kit gating for B/Green encoded Ramos cells with PBMCs.

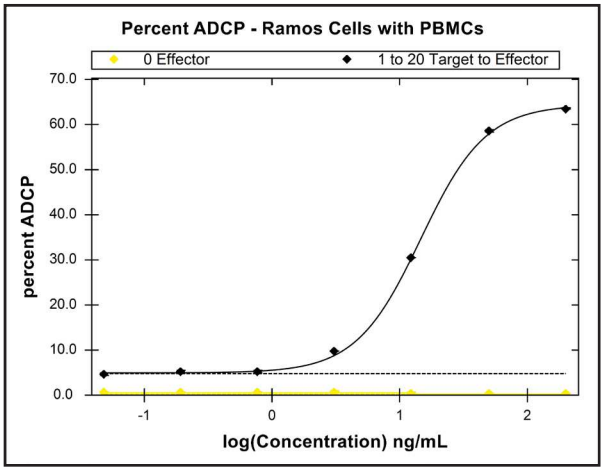

Figure 5: Percent ADCP for 1:20 target-to-effector ratio.

1. Reagent preparation

Thaw frozen **effector cells** and rest overnight in conical tube (5% CO_2, 37°C). Adjust cell density for target-to-effector ratio of 1:20 (2 x 10^6 cells/mL) for PBMCs or 1:5 (5 x 10^5 cells/mL) for purified monocytes.	Rest overnight in conical tube (5% CO_2, 37°C)	☐
Label **target cells** using iQue® Proliferation and Encoding (B/Green) Dye. Adjust cell density to 1 x 10^5 cells/mL.		☐
Prepare dilution series of **test antibody** (customer provided).		☐
Prepare **Human ADCP Detection Cocktail**: Dilute iQue® Cell Membrane Integrity (R/Red) Dye **1:60** into Human ADCP Detection Reagent.		☐

2. Assay protocol

Add **25 µL/well** test antibody to the assay plate.		☐
↓		
Add **25 µL/well** encoded target cells.	Incubate RT, 30 min, Dark	☐
Start time _____ ↓ *Stop time* _____		
Add **25 µL/well** effector cells.	Incubate RT, 60 min, Dark	☐
Start time _____ ↓ *Stop time* _____		
Add **10 µL/well** Human ADCP Detection Cocktail.	Incubate RT, 30 min, Dark	☐
Start time _____ ↓ *Stop time* _____		
Spin at **300 x g for 5 minutes**. Aspirate supernatant. Hard shake (3000 RPM, 60 seconds) residual liquid in plate.		☐
↓		
Add **20 µL/well** Wash Buffer. Acquire data.		☐

Notes:

Sales and Service Contacts

For further contacts, visit www.sartorius.com

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