BioLegend®

Enabling Legendary Discovery™

Antigen Reactive T cell Kit

Catalog Number: 345905

Size: 1 kit (10 tests), ready to use

Reactivity: Human

Configuration: 1 test per pouch, packaged as 10 individual pouches

It is highly recommended that this manual be read in its entirety before using this product.

Do not use this kit beyond the expiration date

For research use only

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Table of Contents

Description

The Antigen reactive T cell kit contains negative control (vial N) and positive control (vial P) in convenient packaging for stimulating human blood or PBMCs along with anti-human LFA-1 PE and surface stain cocktail. This kit contains controls that can be paired with peptides of your choice for detecting antigen specific responses and assessing vaccine responses. An empty 1.4 mL tube is also provided for antigen specific detection for user-provided antigen of choice. Cells or beads stained with individual fluorochrome-conjugated antibodies (PE, PerCP, APC/Cyanine7, FITC, Pacific Blue™) should be used for setting up compensation before running the samples.

The Antigen reactive T cell kit provides a convenient, easy to use, and powerful tool for immunology and vaccine research.

Materials Provided

Description	Quantity	Size	Part #
Negative Control (DMSO)	1 vial per pouch (10 vials/kit)	1 test	750000770
Positive Control (cell activation cocktail)	1 vial per pouch (10 vials/kit)	1 test	750000772
Anti-human LFA-1 PE	3 tubes per pouch (30 tubes/kit)	1 test	750000773
Empty 1.4 mL tube	1 tube per pouch (10 tubes/kit)	1 test	N/A
Surface Stain cocktail*	1 tube per pouch (10 tubes/kit)	5 tests	750000776
RBC Lysis/Fixation Solution (10X)	1 bottle	50 mL	422401
EDTA, 0.08M	1 bottle	0.5 mL	750000774
Cell Staining Buffer	1 bottle	500 mL	420201

**surface stain cocktail contains: Pacific Blue™ anti-human CD8, FITC anti-human CD4, PerCP anti-human CD45, APC/Cyanine7 anti-human CD14, CD41, CD19, CD16 and CD15*

Materials to be Provided by the End User

1. Human blood sample – sodium heparin is recommended.
2. Antigen peptide of interest (2 - 10 µg per stimulation)
3. Pipettes to dispense between 2 µL and 1,000 µL volume
4. Centrifuge
5. Water bath
6. Distilled, deionized water
7. Flow cytometer capable of detecting PE, PerCP, APC/Cyanine7, FITC, and Pacific Blue™ signal.
8. Single-color controls (e.g. Lyse Wash Blood, PBMC or compensation beads) stained with PE, PerCP, APC/Cy7, FITC and Pacific Blue™ for flow cytometer setup
9. For PBMC, use 0.5% BSA solution in Phosphate Buffered Saline (PBS) **containing** 1mM Ca^{2+} and 1mM Mg^{2+} for cell suspension.
10. Floating test tube holder that can hold 1.5mL vials and 5.0mL tubes.

Storage and Handling

1. Store unopened pouches at 2°C - 8°C. They are moisture and light sensitive. We recommend using opened pouches immediately. Please do not store open pouches. Do not use this kit beyond its expiration date.
2. Store other buffers at 2°C - 8°C and use within expiration date.

Experimental protocol

Notes before starting assay:

- *This assay works best with blood collected using sodium heparin as an anti-coagulant and when performed within 3 hours of blood collection. Keep blood at ambient temperature and avoid excessive shaking or mixing.*
- *Cell staining procedure outlined below is for using whole blood as starting material. If fresh blood is not available, it is feasible to do this assay using peripheral blood mononuclear cells (PBMC). Refer to section B below for instructions using PBMCs as starting material.*
    - *We do not recommend using blood stored for longer than 4 hours*
- *Each pouch contains all tubes needed to test one donor. Keep the pouches sealed and unopened until ready to use.*
- *We suggest stimulating using 2 - 10 µg antigen peptide of interest. Further optimization may be required.*

A. Cell Staining Procedure (using whole blood as starting material):

1. Set a water bath to 37°C and make sure it is at 37°C before you start the assay.
2. Dilute required amount of 10x lysis buffer with distilled, deionized water to 1X and make sure it is at room temperature prior to starting the assay. To test one donor, you will need 8 mL of 1X RBC lysis buffer. We recommend to make diluted buffer on the day of the experiment.
3. Remove negative control (N), positive control (P) and the provided empty 1.4 mL vials (for test antigen of interest) from the kit. Centrifuge at 500xg for 2 minutes (or in a picofuge) to ensure contents are at bottom of the tube. Keep at room temperature (RT) for about 3 minutes prior to next step.
4. Add antigen peptide of interest into the provided, empty 1.4 mL vial. Label vial accordingly.
5. Ensure blood samples are evenly mixed, then add 200 µL whole blood to each vial (N, P, and antigen peptide tube in the provided, empty 1.4 mL vial). Cover tubes with caps, do a quick pulse vortex then insert them to a floating test tube holder and incubate at 37°C in the water bath.
    a. To assess **only CD8+ T cell antigen response, incubate at 37°C for 20 minutes**
    b. To detect **CD4 T cell or both CD4 and CD8 T cell antigen response, continue the incubation for 4 hours**.
6. Remove three anti-human LFA-1 PE tubes from 4°C, label them as N for negative control, P for positive control, and T (or equivalent) for test antigen. Centrifuge at 500xg for 2 minutes to ensure the contents are at the bottom of the tube. Keep at room temperature for about 3 minutes.
7. Remove blood-containing tubes from step 5 from water bath, do a quick pulse vortex and store at ambient temperature for 1-2 minutes.
8. Directly transfer the contents from tubes in step 7 to respectively labeled tubes in step 6; vortex and incubate at ambient temperature in the dark for 20 minutes.
9. Add 10 µL EDTA (provided) to each tube, vortex, and incubate at ambient temperature in dark for 10 minutes.

10. Vortex and add 2 mL 1X Fix/Lyse buffer (diluted according to step 2) to each tube then incubate at ambient temperature in the dark for 20 minutes.
11. Centrifuge at 500xg for 5 minutes, decant, vortex to loosen the pellet and add 2 mL cell staining buffer (provided in the kit).
12. Repeat step 11.
13. Centrifuge at 500xg for 5 minutes, decant, vortex to loosen the pellet.
14. Remove a surface stain cocktail tube (SC) from the pouch, centrifuge at 500xg (or in a picofuge) for 2 minutes to ensure the contents at the bottom of the tube.
15. Add 250 µL distilled, deionized water to the surface staining cocktail tube from step 14 and let it dissolve at the ambient temperature in the dark for about 3-5 minutes prior to the next step.
16. Pipette 50 µL reconstituted surface staining cocktail from step 15 to each labeled tube (N, S and P). Vortex and incubate at ambient temperature in the dark for 15 minutes.
17. Wash twice with cell staining buffer as in step 11 and 12. Re-suspend cells in 600 µL – 800 µL cell staining buffer.
18. Acquire sample data using a flow cytometer. For best results, collect at least 35,000 – 55,000 $CD8^+$ gated events. Example gating strategy depicting expected staining pattern is shown below.

B. If using peripheral blood mononuclear cells (PBMCs) as starting material

1. Add antigen peptide of interest into the provided, empty 1.4 mL vial. Label vial accordingly.
2. Isolate PBMC and resuspend in 0.5% BSA in PBS **containing** 1mM Ca^{2+} and 1mM Mg^{2+} to a final cell concentration of 1 x 10^7 cells/mL.
3. Add 200 µL of cell suspension prepared in step 1 above to negative control (N), positive control (P) and antigen peptide (from section B, step 1 above) tubes from the kit.
4. Follow steps 5-18 described in section A to complete the assay.

Data Analysis

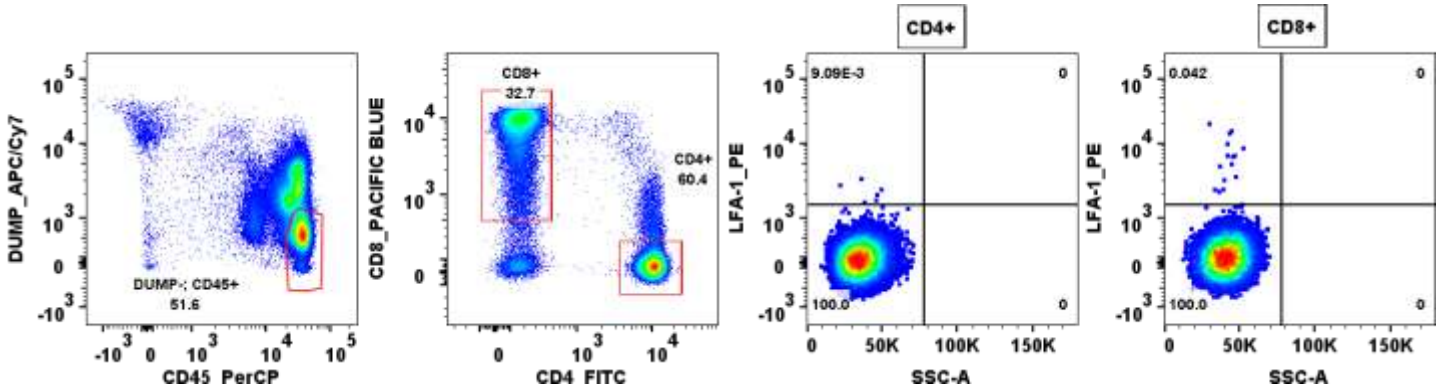
Outlined below is the recommended procedure for data analysis. Steps below were performed on FlowJo™. We recommend adapting and following a similar analysis strategy if using other analysis software.

1. Using the "Create Group" function, group samples based on patient ID; this will help set gates for all samples of the same patient.
2. Once the groups are created in FlowJo™, go to the first group and gate around the lymphocyte population based on the single cells gating (i.e FSC-A vs FSC-H or SSC-A vs SSC-H), then make a dot plot with X-axis representing the compensated PerCP-A (CD45) and Y-axis representing the compensated APC/Cy7-A (Dump).
3. Choose the population that is positive for CD45 and negative for Dump. From that population, create a dot plot with X-axis representing the compensated FITC-A (CD4) and Y-axis representing the compensated Pacific Blue-A (CD8).
4. Gate on two populations
    a. CD4 positive and CD8 negative, label it as CD4+.
    b. CD8 positive and CD4 negative, label it as CD8+.
5. From CD4+ and CD8+ population, make a dot plot with X-axis as FSC-A and Y-axis as compensated PE-A (LFA-1); using the negative control sample (tube N), set a quadrant gate on the positive population and then apply it to all files in that group.
6. Add statistics for percentage of positive, median fluorescence intensity (MFI), event count and any other statistics of interest. Import into an excel file for further analysis using the table editor function.

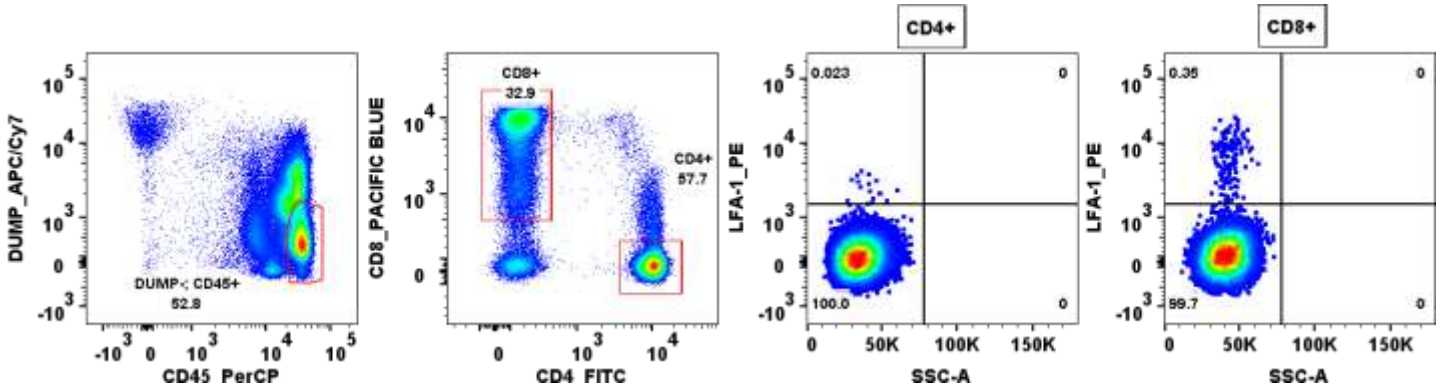
Representative data

Representative data for the use of the Antigen Reactive T cell Kit on whole blood samples collected from a patient who had been vaccinated against SARS-CoV-2. SARS-CoV-2-associated peptide pool was used as the antigen peptide. Flow cytometry gating scheme – CD45+ events were initially gated, followed by specific gating on CD8+ and CD4+ events to identify LFA-1+ cells within each respective population.

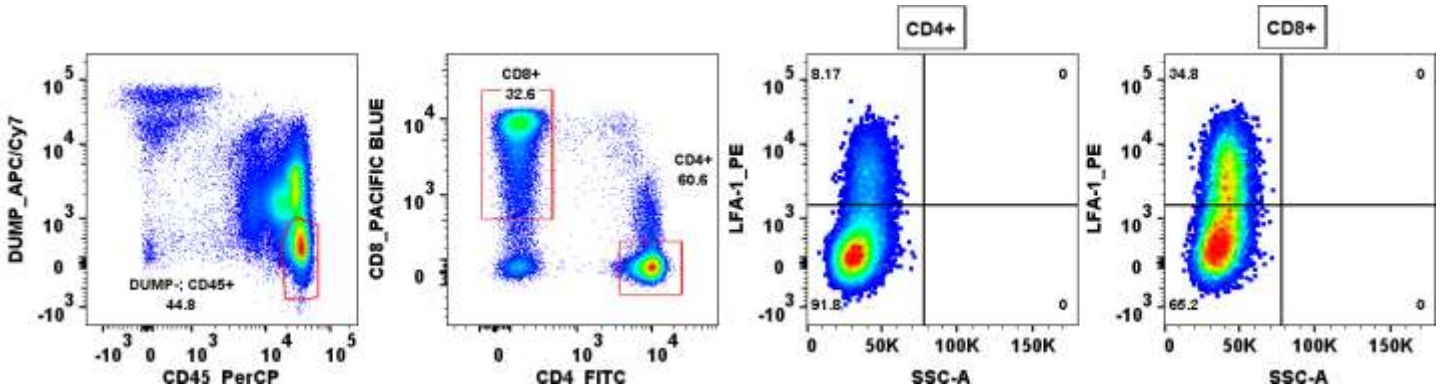
NEGATIVE CONTROL



SARS-CoV-2 (patient sample containing SARS-CoV-2 reactive T cells)



POSITIVE CONTROL (Cell activation cocktail)



Tips for Successful Staining

1. Read the entire manual carefully before the experiment.
2. Plan the experiment in advance. Do not rush any step.
   If possible, do a trial run with 1-2 samples prior to starting a large experiment
3. Make sure that the flow cytometer is well maintained and working well before the experiment.
4. Make sure that there is enough whole blood available for the staining.
5. The experiment should be performed within 3 hours after drawing blood, the earlier the better. If assay cannot be performed the same day as blood collection, PBMC may be enriched and used for this assay. Please plan based on ability to test on same day as blood collection.
7. Make sure to prepare cells for machine setup and compensation.
8. Handle the tubes with care. Do not open pouches or discard desiccant until ready to use.

Frequently Asked Questions

Q: How should the kit be stored?

A: The kit should be stored at 2°C - 8°C upon receipt. Once opened, the tubes can be used or stored at 2°C - 8°C in the pouch and used within a month.

Q: I have added my own antibody solution to the lyophilized product, will the lyophilized antibody work?

A: Yes, as long as the fluorophores on these antibodies are compatible and proper compensation has been applied during acquisition and analysis.

Q: I am not going to use all the reconstituted antibody solution. Can I keep the leftover for later or re-dry the solution in the dark?

A: The antibody is in a one test per tube. There will not be any antibody left if the full test is used. Customers may decide to use less than the recommended volume per test, but this is not recommended and the performance is not guaranteed. Once reconstituted, re-drying is not recommended, as this may result in a loss of signal.

Q: If I don't have enough blood and use less than 150µL, will it still work?

A: The kit may work with lower volume of blood, but we recommend using 200 µL to get the best results. Successful staining has been done with 100 µL of blood per tube using 5 µL instead of 10 µL of EDTA.

Q: Are these tubes made under sterile conditions?

A: The tubes are not sterile. Handle them as you would handle a typical flow cytometry staining protocol or reagent.

Q: Can I use different anti-coagulant than sodium heparin?

A: EDTA and sodium citrate have been successfully used. Please make sure to perform testing within 3 hours of blood collection

Q: Can I add CD3 as a co-stain? Or any other co-stains?

A: Addition of CD3 as a co-stain may result in higher background (due to assay sensitivity). We recommend a trial run prior to adding any other co-stains.

Antigen Reactive T cell Kits are manufactured by **BioLegend**

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For contact information, as well as a list of world-wide BioLegend offices and distributors, please visit: biolegend.com/en-us/contact

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