eBioscience™ Essential Human T-Cell Phenotyping Kit

Catalog Number A42923

Pub. No. MAN0018283 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Invitrogen™ eBioscience™ Essential Human T-Cell Phenotyping Kit is comprised of both positive and negative markers to identify T-cell subtypes:

- **CD3**: The UCHT1 monoclonal antibody reacts with human CD3e, a 20 kDa subunit of the TCR complex. CD3 is expressed by thymocytes in a developmentally regulated manner and by all mature T cells.
- **CD4**: The RPA-T4 monoclonal antibody reacts with human CD4, a 59 kDa cell surface receptor expressed by a majority of thymocytes, subpopulation of mature T cells (T-helper cells) and in low levels on monocytes.
- **CD8**: The RPA-T8 monoclonal antibody reacts with the human CD8a molecule, an approximately 32-34 kDa cell surface receptor in a majority of thymocytes and a subpopulation of mature T cells (Cytotoxic T Cells) and NK cells.
- **CD62L**: The DREG-56 monoclonal antibody reacts with human CD62L, a 76 kDa member of the selectin family and is constitutively expressed on the cell surface of leukocytes.
- **CCR7**: The 3D12 monoclonal antibody reacts with human CCR7 and is a member of the G-protein-coupled chemokine receptor family. CCR7 is expressed on T cells and can be used to distinguish populations of naive from central and effector memory T cells.

Contents and storage

Table 1 eBioscience™ Essential Human T-Cell Phenotyping Kit, (Cat. No. A42923)

Components	Cat. No.	Storage
eBioscience™ Essential Human T-Cell Phenotyping Panel	**A42806**	**2°C to 8°C**
• CD3 (UCHT1)-APC-eF780- Mouse IgG1, kappa • CD4 (RPA-T4) - APC- Mouse IgG1, kappa • CD8 (RPA-T8) - eF450- Mouse IgG1, kappa • CD62L (DREG-56) - FITC- Mouse IgG1, kappa • CCR7 (3D12) - PE - Rat IgG2a, kappa		
eBioscience™ Essential Human T-Cell Phenotyping Isotype Controls	**A42807**	**2°C to 8°C**
• Mouse IgG1, kappa Isotype Control- FITC • Rat IgG2a, kappa Isotype Control-PE		

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Reagents	
eBioscience™ Flow Cytometry Staining Buffer	00-4222-26
eBioscience™ Fixable Viability Dye eFluor™ 506	65-0866-14
UltraComp eBeads™ Compensation Beads	01-2222-42
Instruments and equipment	
12 × 75 mm round-bottom test tubes	MLS
Flow cytometer equipped with at least three lasers (488 nm, 405 nm and 633 nm), with optics capable of detecting fluorophores in the kit.	MLS
Centrifuge (Compatible with 75 mm round bottom test tubes)	MLS
Vortex mixer	MLS
Pipettes	MLS
Refrigerator or ice bucket	MLS
65°C heat block or water bath	MLS
Countess™ II Automated Cell Counter or equivalent counting device	AMQAX1000

Suggested experimental setup

	Laser	488 Laser		633 Laser		405 Laser	
	Fluorophore	**FITC**	**PE**	**APC**	**APC-eFluor™ 780**	**eFluor™ 450 Pac Blue**	**eFluor™ 506**
	Antibody	**CD62L**	**CCR7**	**CD4**	**CD3**	**CD8**	**Viability**
Compensation	Ultracomp-CD62L	CD62L	—	—	—	—	—
	Ultracomp-CCR7	—	CCR7	—	—	—	—
	Ultracomp-CD4	—	—	CD4	—	—	—
	Ultracomp-CD3	—	—	—	CD3	—	—
	Ultracomp-CD8	—	—	—	—	CD8	—
Controls	Cells unstained	—	—	—	—	—	—
	Cells 2 isotypes	mIgG1- FITC	rIgG2a- PE	CD4	CD3	CD8	Viability eFluor™ 506
FMO controls	Cells CD62L FMO	—	CCR7	CD4	CD3	CD8	Viability eFluor™ 506
	Cells CCR7 FMO	CD62L	—	CD4	CD3	CD8	Viability eFluor™ 506
	Cells CD4 FMO	CD62L	CCR7	—	CD3	CD8	Viability eFluor™ 506
	Cells CD3 FMO	CD62L	CCR7	CD4	—	CD8	Viability eFluor™ 506
	Cells CD8 FMO	CD62L	CCR7	CD4	CD3	—	Viability eFluor™ 506
Viability	Cells viability	—	—	—	—	—	Viability eFluor™ 506
Test samples	Cells multiplex	CD62L	CCR7	CD4	CD3	CD8	Viability eFluor™ 506

Perform surface marker staining

Staining can be carried out with either cells in culture or frozen cells directly from thaw. If using frozen cells, thaw according to recommended protocol for the cell type being used.

1. Count cells using a hemocytometer or automated cell counter, such as the Countess™ II Automated Cell Counter.

   Record the cell counts.

2. Collect an appropriate amount of cell suspension from the culture vessel, and add to a sterile 15 mL conical tube.

3. Spin for 5 minutes at 200 × *g* at room temperature and discard the supernatant.

   If the cell type being used has a different recommended centrifugation speed use that setting throughout this protocol.

4. Resuspend cells in Flow Cytometry Staining Buffer so that the concentration is 2×10^5–1×10^6 cells per 100 µL.

   (2×10^6–1×10^7 cells/mL)

5. Aliquot 100 µL of the cells from step 4 into as many tubes (12 × 75 mm tubes) as are needed for experimentation.

   For the T-Cell panel (see "Contents and storage" on page 1), the recommended experimental setup uses 9 samples (see "Suggested experimental setup" on page 2).

6. Positive control for viability (*optional*): Remove 50 µL of the "Viability" sample and place in a 65°C heat block for 15–20 minutes. Afterward, transfer the heat-treated cells back into the same tube with the untreated cells.

   Note: This step is recommended if the percentage of dead cells is expected to be less than 5%. This step allows for visualization of the distinct population of dead cells in order to enable effective gating between live and dead cells.

7. Add 5 µL each antibody or 2 µL CD62L isotype Control (FITC) or 0.625 µL CCR7 isotype control (PE) to the cell suspensions prepared in step 5 and step 6 for the appropriate tubes according to the experimental setup shown in "Suggested experimental setup" on page 2.

8. Add 1 µL of Viability Dye eFluor™ 506 to the samples designated in the eFluor™ 506 Column in "Suggested experimental setup".

9. Briefly vortex all sample tubes. Incubate at 4°C for 30 minutes in the dark.

10. Add 2 mL of Flow Cytometry Staining Buffer, quickly vortex to resuspend the cell pellet, and centrifuge at 200 × *g* for 5 minutes at room temperature. Discard the supernatant.

11. Repeat step 10.

12. Discard supernatant and re-suspend cell pellet in 0.5 mL of Flow Cytometry Staining Buffer.

13. Analyze samples by flow cytometer.

    Vortex each sample before acquisition.

    It is recommended to collect a minimum of 30,000 events for each sample.

    Note: Cells are alive and should be kept on ice and run immediately after completion of staining protocol.

Data acquisition and analysis

Performance Tracking Beads: Startup your Attune™ NxT instrument and software and follow the software prompt to run the performance tracking beads. See *Attune™ Performance Tracking Beads User Guide* (Pub. No. MAN0002636).

Compensation Bead Staining: Follow instructions as per manufacturer's instructions. See ***UltraComp eBeads™ Compensation Beads Technical Data Sheet***.

Typical gating results

Note: All sample plots are generated using FlowJo.

T-cell panel initial gates

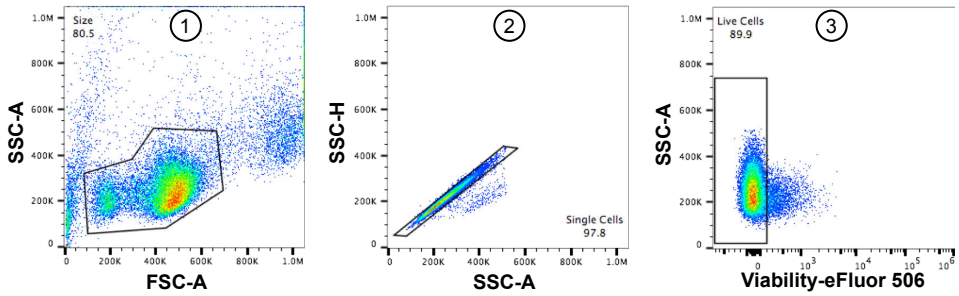

Figure 1 Set gates to exclude unwanted cells

① **Exclude debris**. Parent Gate: Ungated
② **Exclude doublets**. Parent Gate: Size/Debris
③ **Exclude dead cells**. Parent Gate: Single Cells

T-cell panel controls

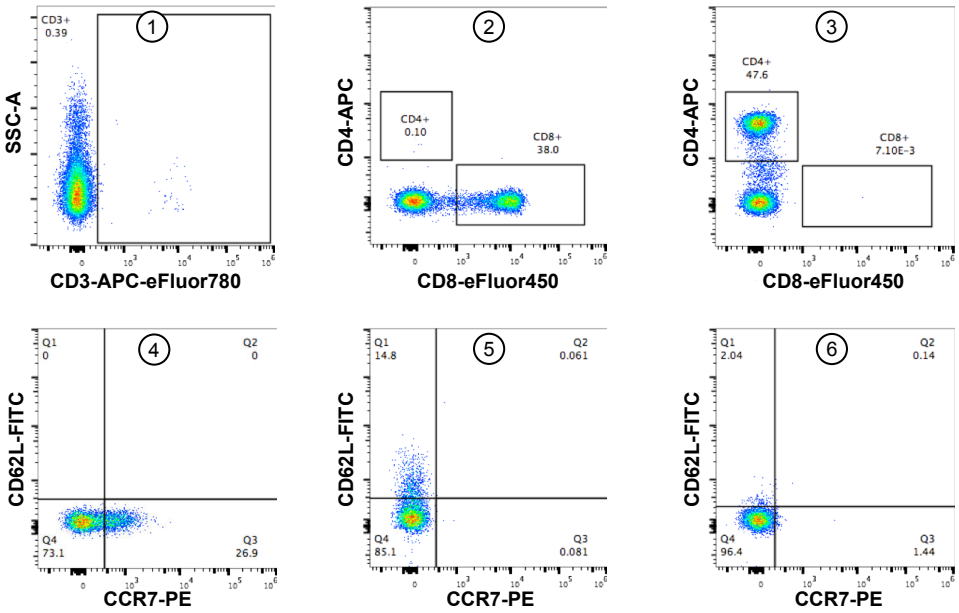

Figure 2 Setting gates with FMO and Isotype Control

① **CD3 FMO**. Parent Gate: Live Cells
② **CD4 FMO**. Parent Gate: CD3+ Cells
③ **CD8 FMO**. Parent Gate: CD3+ Cells
④ **CD62L FMO**. Parent Gate: CD8+ Cells
⑤ **CCR7 FMO**. Parent Gate: CD8+ Cells
⑥ **CD62L and CCR7 Isotype Controls**. Parent Gate: CD8+ Cells

T-cell analysis

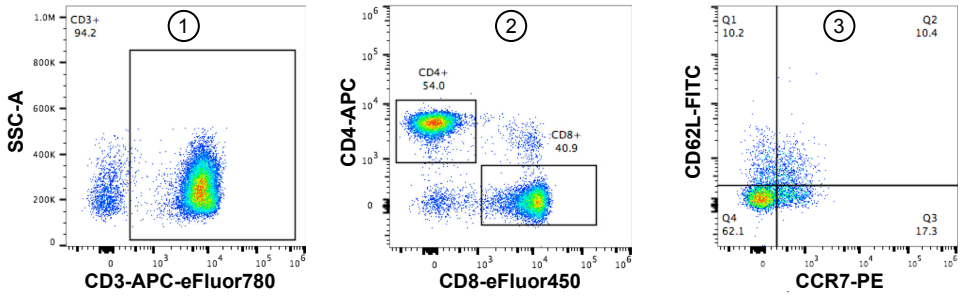

Figure 3 Analysis of cell samples

① **CD3 gating**. Parent Gate: Live Cells

② **CD4 and CD8 gating**. Parent Gate: CD3+ Cells

③ **CD62L and CCR7 gating**. Parent Gate: CD8+ Cells

Limited product warranty

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