DryFlowEx ACT T Screen kit

Cat. No. ED7705

Specificity	CD4	PD-1 (CD279)	HLA-DR	CD3	CXCR5 (CD185)	CD38	CD45	CD8
Clone	MEM-241	EH12.2H7	L243	SK7	J252D4	HIT2	2D1	MEM-31
Isotype (murine)	IgG1	IgG1	IgG2a	IgG1	IgG1	IgG1	IgG1	IgG2a
Fluorochrome	FITC	PE	PerCP-Cy™5.5	PE-Cy™7	APC	APC-Cy™7	Pacific Blue™	Pacific Orange™
λ excitation	488 nm	488 nm	488 nm	488 nm	633 nm	633 nm	405 nm	405 nm
Emission maxima	525 nm	575 nm	692 nm	780 nm	660 nm	780 nm	455 nm	551 nm

Description

DryFlowEx ACT T Screen Kit is designed for Flow Cytometry screening of activated T cells (ACT T) during virus infection.
Multicolor panel of antibody conjugates CD45/ CD8/ CD4/ PD-1/ HLA-DR/ CD3/ CXCR5/ CD38 dried in a single flow cytometry tube enables immunophenotyping of activated T lymphocyte subsets including a specific population of circulating follicular T helper cells (cTfh) in EDTA-anticoagulated human blood.

Reagents provided

ACT T Screen 5T Pack (ED7705—5T) containing 5 pcs of single-test tubes for flow cytometry (12×75 mm).

Cat. No.	Product	Amount
ED7705-5T	ACT T Screen 5T Pack	10 packs

ACT T Screen Compensation Tubes Pack (ED7705—CT) containing 8 pcs of single-test compensation tubes for flow cytometry (12×75 mm).

Cat. No.	Product	Amount
ED7705-CT	ACT T Screen Compensation Tubes Pack	1 pack
ED7705-CT1	CD4 FITC	1 pc
ED7705-CT2	CD279 (PD-1) PE	1 pc
ED7705-CT3	HLA-DR PerCP-Cy™5.5	1 pc
ED7705-CT4	CD3 PE-Cy™7	1 pc
ED7705-CT5	CD185 (CXCR5) APC	1 pc
ED7705-CT6	CD38 APC-Cy™7	1 pc
ED7705-CT7	CD45 Pacific Blue™	1 pc
ED7705-CT8	CD8 Pacific Orange™	1 pc

Materials required but not provided

Ultrapure, deionized water
Pipettes and dispensable pipette tips
Lysing solution EXCELLYSE Easy (cat. No. ED7066)

Storage and handling

The product ED7705 retains its functional characteristics when stored at laboratory temperature until the specified expiry date printed on product label.
Use immediately after opening. Do not reuse.
Do not expose to direct sunlight. Do not freeze the product.
Do not use the product after the expiry date printed on product label.
ALU pouches containing 5 product test tubes (ED7705-5T) must be opened directly before use. Reseal unused ED7705-5T product tubes in the ALU pouch containing drying silicagel using the pouch zip-lock.
Once the ALU pouch ED7705-5T is opened, use the remaining single-test tubes within the period of one month.

Warnings and precautions

Intended for research use only.
Blood samples are considered as potentially infectious and must be handled with care. Use protective gloves and follow procedures for handling potentially infectious materials. Avoid contact of human blood samples with skin, eyes and mucous membranes. It is highly advised to close test tube using tube caps to minimize risk of exposure to infectious specimen.
Do not use specimen with signs of hemolysis.
A flow cytometer equipped with violet (405 nm), blue (488 nm) and a red (640 nm) laser with appropriate fluorescence filters and detectors must be used for sample analysis. The flow cytometer must be maintained and calibrated on a regular basis for stable fluorescence detector sensitivity.
Improperly compensated multiparameter flow cytometry data may be interpreted incorrectly. A set of compensation tubes (Cat. No. ED7705-CT) is a part of the product. These tubes contain dried single-color antibodies (8 compensation tubes in separate ALU pouch), which are also part of the dried multiparameter panel in a Test tube and provide positive and negative cell population when used for staining. Compensation tubes must be handled the same way as Test tubes.
Any application non-compliance may affect test results.

Application

Specimen

Use peripheral whole blood collected into a sterile tube containing anticoagulant (EDTA).
Store the collection tube at room temperature prior to staining.
The specimen must be stained within 48 hours after collection.

Lysing solution preparation

Prepare freshly diluted 1x EXCELLYSE Easy (Cat. No. ED7066). Lysing solution is provided as a 10× concentrate and must be diluted 10× using deionized water prior to use (1 part of reagent into 9 parts of water). Prepare 2ml of diluted lysing solution per 1 tube. Assign with the date of preparation and store at 2 - 25 °C for maximum of 4 weeks.

Test tube and compensation tube preparation

1. Add 100 µl of peripheral whole blood to the Test tube or the Compensation tube.
2. Vortex excessively for **a minimum of 7 seconds. Note:** Shortening the vortex time may negatively affect test results
3. Incubate the tube for 20 minutes at room temperature in the dark.
4. Lyse the erythrocytes by adding 2 ml of 1x EXCELLYSE Easy to the tube.
5. Incubate the tube for 10 minutes at room temperature in the dark.
6. Centrifugue the tube for 5 minutes at 300×g.
7. Discard supernatant and resuspend the cell pellet using 0.2 – 0.5 ml PBS.
8. Analyze the sample within 2 hours after staining.

Cytometer set-up

Set the flow cytometer fluorescence detector voltages using Euroflow® protocol by Rainbow calibration particles, 8 peaks (Spherotech Inc., Cat. No. RCP 30-5A (Euroflow)). BD FACS cytometers may be set using BD™ CS&T Beads. Beckman Coulter cytometers may be set using Flow-Set Pro Fluorospheres..

- van Dongen JJ et al., 2012, Leukemia
- https://www.spherotech.com/Calibration Particles.htm

Set „Protocol“ for Beckman Coulter flow cytometers and „Experiment“ for BD flow cytometers to acquire data from detectors:

- Forward Scatter (signal height)
- Forward Scatter (signal area)
- Side Scatter (signal area)
- FITC (FL1 for Beckman Coulter Navios®)
- PE (FL2 for Beckman Coulter Navios®)
- PerCP-Cy™5.5 (FL4 for Beckman Coulter Navios®)
- PE-Cy™7 (FL5 for Beckman Coulter Navios®)
- APC (FL6 for Beckman Coulter Navios®)
- APC-Cy™7 (FL8 for Beckman Coulter Navios®)
- Pacific Blue™ (FL9 for Beckman Coulter Navios®)
- Pacific Orange™ (FL10 for Beckman Coulter Navios®)

Prepare a compensation matrix to eliminate fluorescence spillover or use compensation matrix prepared from the same lot of compensation tubes ED7705-CT. Follow your flow cytometer manufacturers instructions.
Compensation tubes must be prepared the same way as test tubes (See Test tube and Compensation tube preparation).
After setting a compensation Experiment, measuring Compensation tubes and selecting positive and negative cell events BD FACSDiva™ software generates compensation matrix automatically.
In Navios software it is required to compensate spillover between required fluorescence detectors manually using “sliders“ in compensation mode.

Flow Cytometry Analysis

1. Set threshold („Discriminator“ for Navios® cytometers) on event size (Fig. 1), or Fluorescence Intensity in Pacific Blue™ detector (FL9) (Fig. 2), or both, so that **minimum 90 %** of all recorded events are leukocytes. Very high Threshold / Discriminator value settings may cause irreversible loss of lymphocyte events from analysis

Figure 1. *Depiction of sufficient event size Threshold settings in a Forward vs. Side Scatter dot-plot*

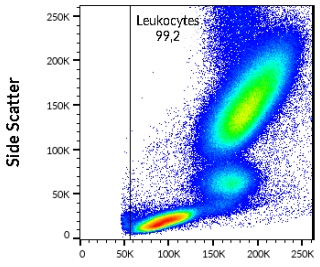
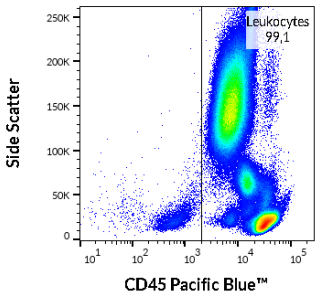


Figure 2. *Depiction of sufficient fluorescence intensity Threshold settings in a CD45 Pacific Blue™ (FL9) vs. Side Scatter dot-plot*



2. Acquire **minimum of 200 000** events. Due to overall low percentage of target cell population it is of most importance to acquire highest amount of leukocyte events possible.

Data analysis and phenotyping procedure

Analyze measured data in FlowJo software, BD FACSDiva™ or Navios EX acquisition SW. Check for proper fluorescence spillover compensation. Non-compensated data may be compensated after data acquisition after applying pre-calculated or saved compensation matrix.
Identify ACT T subsets (target cell populations) according to Phenotyping strategy, see Appendix No. 1.

Data interpretation

Percentage of ACT T subsets from all CD3⁺ T cells (Fig. 3 - 5) may change during a viral infection.

An increase in ACT T subsets percentage may be detected with the viral infection onset. This percentage is expected to change further as the viral infection proceeds..

Figure 3. *Flow cytometry dot-plot depiction of target population - CD4⁺ CXCR5⁺ PD-1⁺ circulating follicular T helper cells in normal human peripheral whole blood labeled using ED7705-5T.*

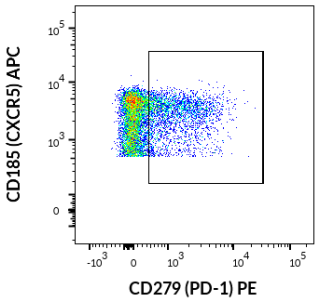


Figure 4. *Flow cytometry dot-plot depiction of target population - CD4⁺ CD38⁺ HLA-DR⁺ activated T cells in normal human peripheral whole blood labeled using ED7705-5T.*

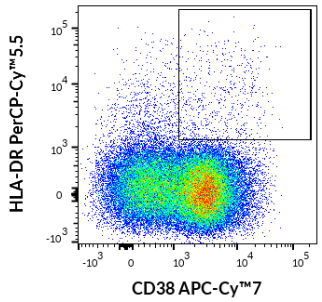
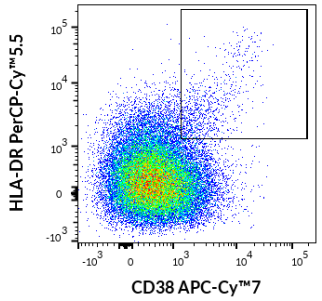


Figure 5. *Flow cytometry dot-plot depiction of target population - CD8⁺ CD38⁺ HLA-DR⁺ activated T cells in normal human peripheral whole blood labeled using ED7705-5T.*



Literature

This product and its use have not been published yet.

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Trademarks

- BD FACS, BD™ CS&T Beads, BD FACSDiva™ and FlowJo® are registered trademarks of Becton Dickinson
- Navios® is a registered trademark of Beckman Coulter

Revision history

- Version 3

Symbols

REF	Catalog number
LOT	Batch code
	Use-by date
	Temperature limits
	Consult instructions for use
	Keep away from sunlight
	Manufacturer
RUO	For Research use only. Not for use in diagnostic or therapeutic procedures.

DryFlowEx ACT T Screen kit

50×1 tests | Cat. No. **ED7705**

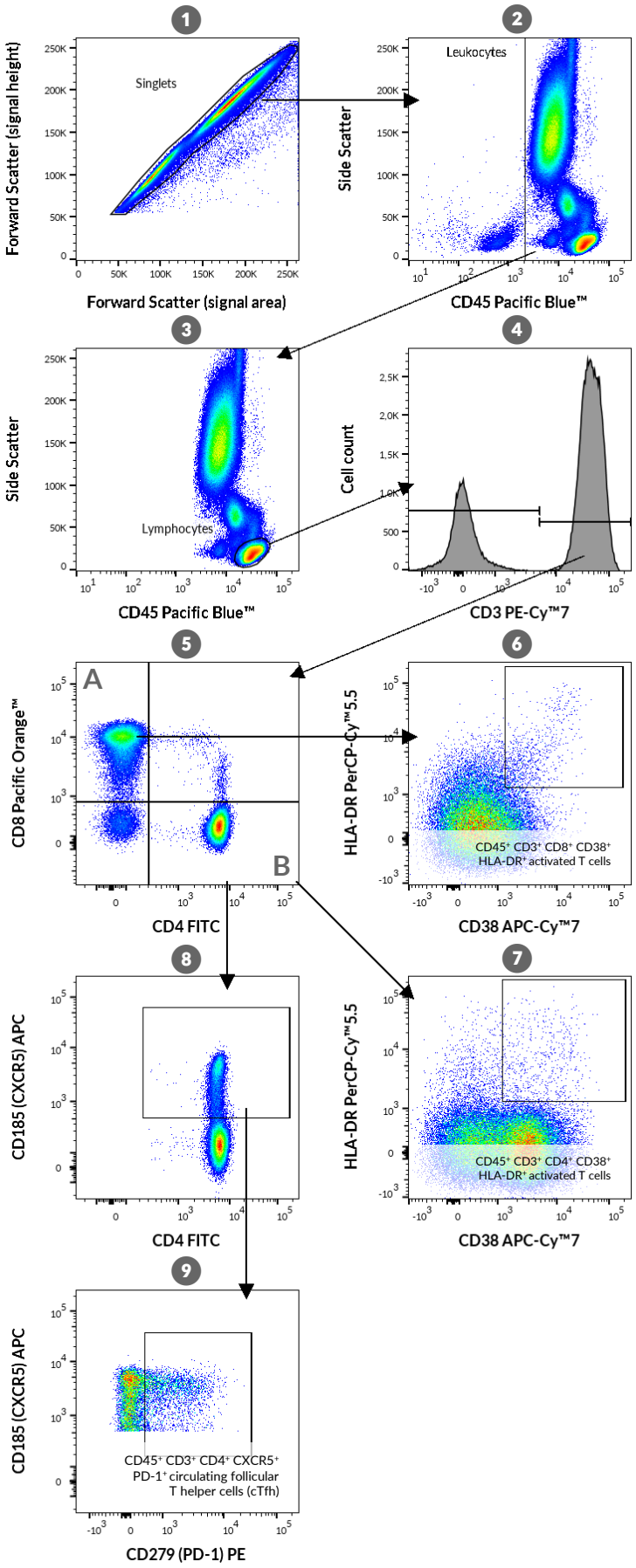
For Research use only.
Not for use in diagnostic or therapeutic procedures.

Technical Data Sheet

Version: ED7705_TDS_v3_EN
Date of Issue: 23-04-2020

EN

Appendix No. 1 – Phenotyping strategy



1. Singlet events
2. $CD45^+$ singlets - leukocytes
3. $CD45^+$ SSC^{low} lymphocytes
4. $CD45^+CD3^+$ T cells
5. **A:** $CD45^+$ $CD3^+$ $CD8^+$ T cells (cytotoxic)
   B: $CD45^+$ $CD3^+$ $CD4^+$ T cells (helper)
6. Target population - $CD45^+$ $CD3^+$ $CD8^+$ $CD38^+$ $HLA\text{-}DR^+$ activated T cells
7. Target population - $CD45^+$ $CD3^+$ $CD4^+$ $CD38^+$ $HLA\text{-}DR^+$ activated T cells
8. $CD45^+$ $CD3^+$ $CD4^+$ $CXCR5^+$ T cells
9. Target population - $CD45^+$ $CD3^+$ $CD4^+$ $CXCR5^+$ $PD\text{-}1^+$ circulating follicular T helper cells (cTfh)