T-cell BlastoFlowEx Kit

Cat.No. ED7642

Description

T-cell BlastoFlowEx Kit is designed to measure the proliferative response of T-lymphocytes in activated samples of whole human blood. The kit utilizes anti-CD3 / anti-Ki67 antibody cocktail to detect the proliferating lymphocytes.

Specification

Fix and Lysing Solution (10x concentrated) contains solution of fixation-lysing reagent.
Permeabilizing Solution (10x concentrated) contains solution of permeabilizing reagent.
EDTA (Ready to use) contains buffered solution of divalent ions chelating reagent.
CD3 APC / Ki-67 PE (Ready to use) contains antibody cocktail in stabilizing buffer (see the table blow).

Content	2 x 2,5 ml	
Usage	50 µl per test	
Specificity	CD3	Ki-67
Clone	MEM-57	Ki-67
Isotype (mouse)	IgG2a	IgG1
Fluorochrome	APC	R-PE
λ excitation	633 nm	488 nm
Emission maximum	660 nm	575 nm

Reagents provided

ED7642-1 Fix and Lysing Solution, 1 x 25 ml, intended to prepare 250 ml of 1x solution (2 ml per test).
ED7642-2 Permeabilizing Solution, 1 x 10 ml, intended to prepare 100 ml of 1x solution (0.5 ml per test).
ED7642-3 EDTA, 1 x 2.5 ml (0.025 ml per test)
ED7642-4 CD3 APC/Ki-67 PE, 2 x 2.5 ml (0.05 ml per test).
The content of the kit is sufficient for 100 staining reactions.

Materials required but not provided

Deionized water (dH_2O)
Phosphate buffered saline (PBS)
5ml test tubes (12 × 75 mm)

Storage and handling

Store the T-cell BlastoFlowEx Kit at 2-8 °C. Expiration date is printed on each reagent label and on the outer packaging label.
Shelf life after the first opening is not different from the shelf life printed on labels.

Warnings and precautions

- Intended for research use only.
- Do not use reagents after their expiration date.
- Avoid contamination of reagents.
- Avoid prolonged exposure to light.
- Do not freeze.
- **Fix and Lysing Solution** and **Permeabilizing Solution** contain formaldehyde, methanol, and diethylene glycol.
  The solutions are classified as hazardous according the Regulation (EC) No 1272/2008. Wear protective gloves, protective clothing, eye protection and face protection when working with the reagents.
  H phrases
  H302+312+332: Harmful if swallowed, in contact with skin or if inhaled.
  H315: Causes skin irritation.
  H317: May cause an allergic skin reaction
  H319: Causes serious eye irritation.
  H335: May cause respiratory irritation.
  H351: Suspected of causing cancer
  H371: May cause damage to organs.
  H373: May cause damage to organs (kidney) through prolonged or repeated exposure if swallowed.
  P phrases
  P270: Do not eat, drink or smoke when using this product.
  P280: Wear protective gloves / protective clothing / eye protection / face protection.
  P301+P312: IF SWALLOWED: Call a POISON Center or doctor/physician if you feel unwell.
  P302+P352: IF ON SKIN: Wash with plenty of soap and water.
  P305+P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- **EDTA** reagent contains Ethylenediamine tetraacetic acid·Na2-salt.
  The solution is classified as dangerous according the Regulation (EC) No 1272/2008.
  H phrases
  H373: May cause damage to organs (kidney) through prolonged or repeated exposure if swallowed.
  P phrases
  P260: Do not breathe dust/fume/gas/mist/vapours/spray.
  P314 Get medical advice/attention if you feel unwell.
  P501: Dispose of contents/container to authorized facility for dangerous wastes.
- See product Safety Data Sheet for full information on the potential hazards and how to work safely with the product.
- 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.
- Blood for stimulation must be collected into a tube containing heparin. Anticoagulants EDTA and citrate negatively affect the stimulation response.
- The flow cytometer should be calibrated on a routine basis using fluorescent microbeads to ensure the stable sensitivity of detectors.
- Flow cytometer may produce false results if the device has not been regularly calibrated and maintained appropriately.

Application

Measurement the proliferative response of T-lymphocytes

Whole blood stimulation in tubes

NOTE: The kit was optimized for use with whole blood cultures stimulated inside 12 x 75 mm tubes. The stimulation tubes containing dried mitogens are available from EXBIO: Cat.No. ED7634 (PHA), Cat.No. ED7635 (PWM), Cat.No. ED7636 (Con A), Cat.No. ED7637 (Stimulation Negative Control), Cat. No. ED7673 (CD3/CD28).

1. Place a mitogen containing tube inside a laminar flow cabinet. Remove the cap, do not discard it, keep it inside the cabinet.
2. Add 0.5 ml of culture medium to the tube.
3. Add 50 µl of blood (heparin anticoagulated).
4. Cap the tube and vortex gently to mix the content.
5. Ensure the cap ventilation position and place the capped tubes in a cell incubator for 3 days (37 °C with 5% CO2).

Detection of Ki67 expressing T-lymphocytes with T-cell BlastoFlowEx kit

Reagent preparation

Fix and Lysing Solution:
Dilute 10x in dionized water. Prepare **2 ml** of the diluted solution for each staining reaction.

Permeabilizing solution:
Dilute 10x in deionized water. Prepare **0.5 ml** of the diluted solution for each staining reaction.

Required for handling

Cylinders and beakers to dilute the reagents
Vortex mixer
Automatic pipettes with disposable tips
Centrifuge with rotor for 5ml tubes
Waste container with disinfectant to collect the supernatants after cell centrifugations
Flow cytometer - blue laser excitation 488 nm and 633 nm and proper filters.

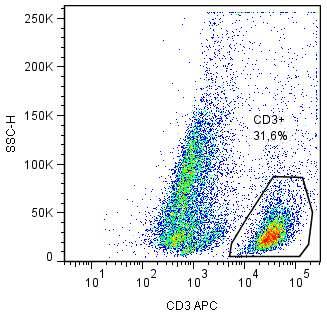
Procedure

1. Remove the tubes from the incubator.
   Remove and discard the caps.
   Add **25 µl of EDTA** solution into each tube and mix.
2. Incubate for 10 minutes at 37 °C.
3. Add 2 ml of PBS, mix.
   Centrifuge the cells for 5 minutes at 400 g.
   Decant supernatant.
4. Shake the tubes a little to disturb the pellet.
   Add **2 ml of the diluted Fix and Lysing Solution**, mix.
   Incubate for 10 minutes at room temperature.
5. Centrifuge the cells for 5 minutes at 400 *g*.
   Decant supernatant.
6. Add **0.5 ml of the diluted Permeabilizing Solution**, mix.
   Incubate for 10 minutes at room temperature.
7. Add 2 ml of PBS.
   Centrifuge the cells for 5 minutes at 400 *g*.
   Decant supernatant.
8. Add **50 µl of CD3/Ki-67 PE** antibody cocktail, mix.
   Incubate for at least 30 minutes at room temperature in the dark.
9. Add 2 ml of PBS.
   Centrifuge the cells for 5 minutes at 400 *g*.
   Decant supernatant.
10. Resuspend the cells in 0.1-0.3 ml of 1% formaldehyde in PBS or Fix and Lysing solution diluted in PBS (mix 1 part of Fixation Buffer with 9 parts of PBS).
    Store the processed samples at 2-8 °C in the dark until analysis.

Flow Cytometric Analysis

Set the voltage on **light scatter detectors**, forward light scatter and side (perpendicular) light scatter so that the events of interest are on scale (consider that stimulated and unstimulated samples differ in their properties).
Set the threshold on **forward light scatter** so that only cells of interest are recorded and most of the debris excluded. Do not set threshold on APC fluorescence, in our experience this setting causes distortion of results due to the inappropriate fluorescence signal/background processing.
Set voltage on **fluorescence detectors** so that all events of interest are on scale.
Adjust the forward scatter area scaling factor (BD instruments) to enhance singlets vs doublets discrimination. When this factor is set properly the FSC peak area and the FSC peak height will have the same values and the singlet events will form a diagonal line (45 degrees, passing through zero).
Plot the ungated events as side scatter vs CD3 APC fluorescence. Draw a gate around CD3+ events (Figure 1).
Acquire at least 3,000 of CD3+ lymphocytes per sample.

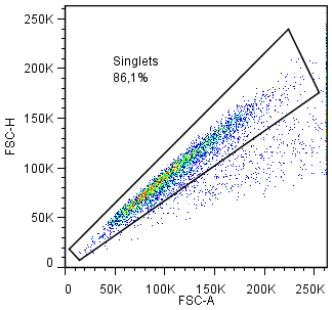
Fig. 1 Gate for CD3+ cells (PHA stimulation).



Analysis of samples

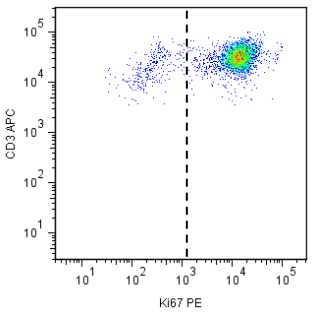
Plot the events from CD3+ gate as light forward scatter signal peak height vs light forward scatter signal peak area. Draw a diagonal gate around singlets (Figure 2).

Fig. 2 Gates for Singlets (PHA stimulation).



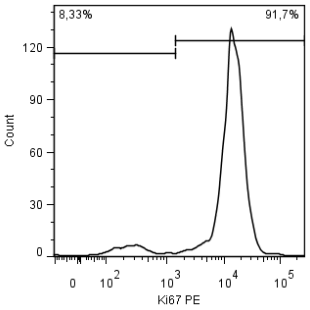
Check the distribution of Ki-67 signal by displaying the CD3+/singlets as a dot plot with CD3 APC signal on the Y axis vs Ki-67 PE signal on the X axis. Look for the best discrimination value between the negative and positive population (dashed line) (Figure 3).

Fig. 3 Ki-67 fluorescence (PHA stimulation).



Display the CD3+/singlets in histogram of PE fluorescence. Draw the discrimination line between the negative and positive peaks. Apply to all tubes and evaluate the Frequency of CD3+Ki-67+ percentage (Figure 4).

Fig. 4 Frequency quantitation (PHA stimulation).



References

n/a

Manufacturer

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Trademarks

n/a

Revision History

• Version 1, ED7642_TDS_v1
Initial Release
• Version 2, ED7642_TDS_v2
Typing errors correction.
• Version 3, ED7642_TDS_v3
Recommended centrifugation forces within procedure changed from 300 *g* to 400 *g*. Mobilisitaion of the cell pellet recommendation added to the step 4 of the procedure.
• Version 4, ED7642_TDS_v4
The supplied amount of Permeabilizing Solution changed from 6 ml to 10 ml.
• Version 5, ED7642_TDS_v5
The company logo changed. IFU layout changed. Manufacturer postal code changed from 25242 to 25250. Test principle, most of the text about blood stimulation and the corresponding required materials for stimulation is omitted from the IFU. The example of the negative stimulation control sample analysis is not shown.
• Version 6, ED7642_TDS_v6
Warnings and precautions updated.
• Version 7, ED7642_TDS_v7
Typing errors correction in CS version.

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.

T-cell BlastoFlowEx Kit

100 tests | Cat.No. **ED7642**

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

Technical Data Sheet

Version ED7642_TDS_v7_EN
Date of Issue: 28-09-2021

EN

The product is intended For Research Use Only. Diagnostic or therapeutic applications are strictly forbidden.
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