CellCycleFlowEx Kit

Cat.No. ED7069

Description

CellCycleFlowEx Kit is intended for cell cycle analysis using flow cytometry. The kit is suitable for testing suspensions of isolated cells, such as leukocytes isolated from peripheral blood (PBMC) or cells from tissue culture.
Proliferating cells duplicate their DNA during the cell cycle. The DNA is subsequently segregated equally into the daughter cells. Measurement of cellular DNA content within a proliferating cellular population recognizes the cells in S phase (actively duplicating their DNA), G2/M phase (having double the amount of DNA then resting cells) and G0/G1 phase (the same amount of DNA as resting cells) of the cell cycle. Analysis of the percentage of cells at different stages of the cycle according to their DNA content is used to assess the proliferative response to stimulation with mitogens (lymphocyte transformation test) or to monitor the effect of cell cycle inhibitors.
The tested cells are washed and resuspended in a buffer to achieve a homogeneous suspension. Then the cells are fixed and permeabilized with 70% ethanol. Subsequently the cellular DNA is stained with propidium iodide, which passes through the permeabilized membrane and intercalates between the bases of nucleic acid. Propidium iodide binds to the DNA stoichiometrically, which means that the amount of bound propidium iodide is proportional to the amount of DNA within the cell. Labeling of DNA takes place in buffer with RNase which digests the contaminating cellular RNA. After laser excitation the cells emit fluorescence of which intensity is proportional to the DNA content. Flow cytometric analysis of the cell suspension distinguishes the cells in G0/G1, S and G2/M phase of the cell cycle.

Specification

RNase A – 2 vials, each contains 1 ml of RNase A solution. The amount of the reagent in each vial is sufficient for 100 tests (Ready to use).
Propidium Iodide – 1 vial, contains 2 ml of propidium iodide solution. The amount of the reagent is sufficient for 200 tests (Ready to use).
10x Wash Buffer – 2 bottles, each contains 30 ml of 10x concentrated solution. The amount of the reagent in each bottle is sufficient for 100 tests.

Reagents provided

ED7069-1 **RNase A**, 1.0 ml
ED7069-2 **Propidium Iodide**, 2.0 ml
ED7069-3 **10x Wash Buffer**, 30 ml
The content of the kit is sufficient for 200 tests.

Materials required but not provided

5ml test tubes (12 × 75 mm)
70% ethanol at least of p.a. purity
Ice bath / container with crushed ice

Storage and handling

CellCycleFlowEx Kit consists of two parts:
a) Box with 10x Wash Buffer and Propidium iodide. Store the box at 2-8 °C.
b) Resealable bag with RNase A vials.
Store the bag with vials at -20 °C.
Expiration date is printed on reagent labels and on the kit outer packaging label.

Warnings and precautions

- Intended for research use only.
- Do not use reagents after the expiration date.
- Avoid contamination of reagents.
- Protect the reagent Propidium Iodide from light.
- Wear protective gloves.
- The flow cytometer should be calibrated on a routine basis using fluorescent microbeads to ensure the stable sensitivity of detectors.
- There is a risk of misinterpretation of the data if the singlet gate and the G0/G1, S and G2/M phase borderlines were not placed correctly.
- Any deviation from the recommended procedure can affect the test results.
- In case of reagents deterioration or if data obtained show any performance alteration, please contact manufacturer using following e-mail address: technical@exbio.cz

Required for handling

Automatic pipettes with disposable tips
Vortex mixer
Centrifuge with rotor suitable for 5ml tubes
Flow cytometer - blue laser excitation 488 nm and proper filters

Procedure

Preparation of reagents before the assay

1. Dilute **10x Wash Buffer** with deionized water (1 part of 10x Wash Buffer and 9 parts of deionized water) and keep it cold at 2-8 °C. Store the diluted solution (**1x Wash Buffer**) at 2-8 °C for up to 6 months.

   10x Wash Buffer may contain precipitated salts. If present, place the bottle to room temperature or into a water bath set to 37 °C and wait till the salts dissolve. Mix to ensure homogeneity before dilution.
2. Prepare **PI staining solution** (0.5 ml per test) according to the following formulation:
   1x Wash Buffer 1 ml
   Propidium iodide 0.02 ml
   RNase A 0.02 ml
   The solution may be stored up to 2 weeks at 2-8 °C.
3. Prepare **70% ethanol** (not provided) in deionized water and keep it cool at -15 to -30 °C.

Washing the cells

1. Centrifuge the cells at 300 g for 5 minutes at laboratory temperature. Remove the supernatant.
2. Resuspend the cells in 1 ml of cold 1x Wash Buffer (2-8 °C).
3. Centrifuge the cells at 300 g for 5 minutes at laboratory temperature. Remove the supernatant.
   Resuspends the cells in cold 1x Wash Buffer (2-8 °C) to concentration 2.5-10 x 10^6 cells/ml.
   For one test you need 200 µl of cell suspension.

Ethanol fixation

4. Pipette 200 µl of the cell suspension to a 5 ml test tube and place it on ice.
5. Add dropwise 2 ml of cold 70% ethanol (-15 to -30 °C) while continuously mixing (vortexing) the tube. Then immediately place the tube back to ice.
   The procedure may be stopped at this step. The fixed cells can be transported or stored at 2-8 °C for up to 2 weeks.

Staining of DNA

6. Centrifuge the cells at 300 g for 5 minutes at laboratory temperature. Remove the supernatant.
7. Resuspend the cells in 1 ml of cold 1x Wash Buffer (2-8 °C).
8. Centrifuge the cells at 300 g for 5 minutes at laboratory temperature. Remove the supernatant.
9. Resuspend the cells in 0.5 ml of PI staining solution and incubate for 30 minutes at room temperature in the dark.
10. Thoroughly vortex the samples and measure with flow cytometer within 4 hours.
    The cells in PI staining solution form aggregates in time. They need to be resuspended by vortexing prior the flow cytometric analysis.

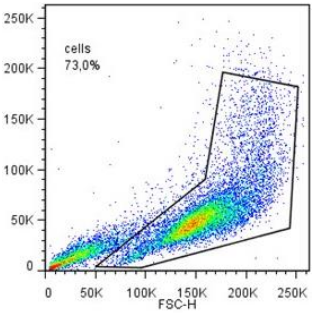
    The level of DNA labeling increases in time thus the samples measured in 30 minutes time and those measured in 4 hour time differ in their G1 peak mean fluorescence of about 10% and the latter have higher G2/G1 fluorescence ratio.

Flow Cytometric Analysis

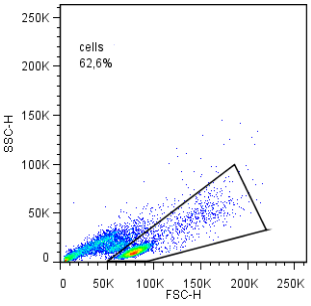
Analyze stained samples using flow cytometer. Acquire at least 20,000 events per sample. For precise measurements we recommend setting the flow at the lowest rate. High speed of acquisition negatively affects the resolution of the populations.
Visualize the measured data in the side-scatter (**SSC**) versus forward scatter (**FSC**) dot-plot. Set a gate around the target cell population (gate in Figure 1).

Figure 1 Delimitation of the target cell population:

a) PBMC stimulated with PHA (5 µg/ml) for 3 days



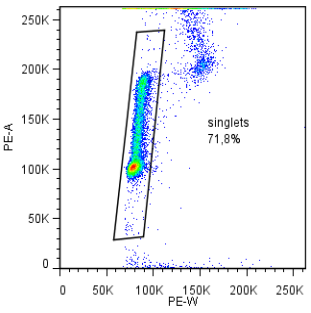
b) unstimulated controls



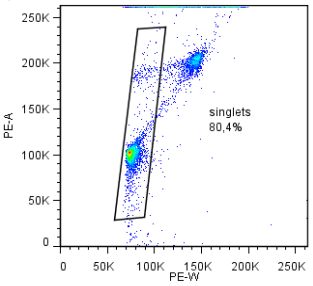
Display the target population in the PE-Width (PE-W) versus PE-Area (PE-A) and draw a gate containing singlets to eliminate cell aggregates from further analysis (Figure 2).

Figure 2 Delimitation of singlets:

a) stimulated PBMCs



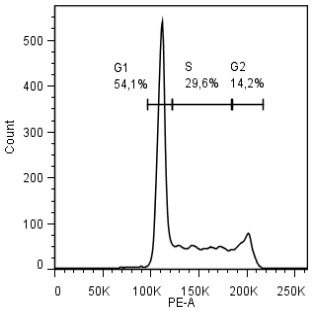
b) controls



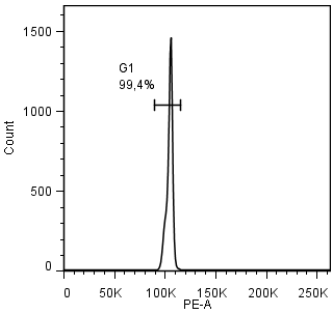
Display singlets in the histogram, where the X axis represents the fluorescence intensity in PE channel in linear scale (Figure 3). Set the PMT in PE channel to ensure the G1 peak is positioned in at least one third of the scale, see Figure 3.
Set borderlines between the G0/G1, S and G2/M phases of cell cycle, as shown in Figure 3. Calculate the proportion of cells in each phase. Alternatively, use suitable software (e.g., FlowJo) that can provide cell cycle analysis automatically.

Figure 3 Distribution of cells according to the DNA content into the G0/G1, S and G2 / M phases of the cell cycle :

a) PHA stimulated PBMCs



b) controls



References

Pozarowski P, Darzynkiewicz Z. Analysis of cell cycle by flow cytometry. Methods Mol Biol. 2004;281:301-11.

Manufacturer

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Trademarks

N/A

Revision History

• Version 1, ED7069_TDS_v1
Initial Release.
• Version 2, ED7069_TDS_v2
Figures 1, 2 and 3 were interchanged.
• Version 3, ED7069_TDS_v3
The company logo changed. TDS layout changed. Manufacturer postal code changed from 25242 to 25250.

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.

CellCycleFlowEx Kit

200 tests | Cat.No. **ED7069**

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

Technical Data Sheet

Version ED7069_TDS_v3_EN
Date of Issue: 26-05-2021

EN

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