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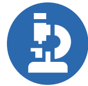 Laborgeräte & Service

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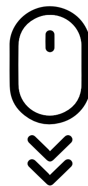
See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Elabscience®

8th Edition, revised in May, 2020

（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Cell Cycle Assay Kit (Fluorometric-Red)

Catalog No: E-CK-A351

Sizes: 20 Assays / 50 Assays / 100 Assays

产品编号	产品名称	20 Assays	50 Assays	100 Assays	Storage
E-CK-A351A	RNase A Reagent	1 mL×2	5 mL	10 mL	-20 ℃
E-CK-A351B	Propidium Iodide(PI) Staining Solution	8 mL	10 mL×2	10 mL×4	2~8 ℃
Manual		**One Copy**			

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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Introduction

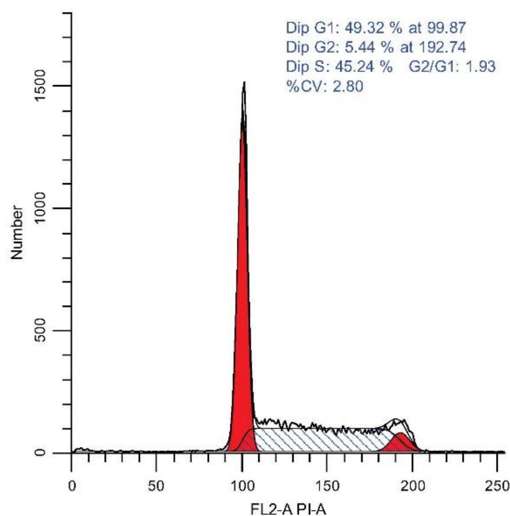
Elabscience® Cell Cycle Assay Kit (Fluorometric-Red) is a kit that detects cell cycle by detecting DNA content.

Detection Principle

Cell cycle refers to the whole process from the end of one mitosis to the end of the next. During this process, the genetic material is replicated and doubled, and evenly distributed to two daughter cells at the end of division. Cell cycle can also be divided into phases like interphase and Metaphase. Intercellular phase can also be divided into dormancy (zero gap, G0), prophase of DNA synthesis (first gap, G1), anaphase of DNA synthesis (synthesis, S) and anaphase of DNA synthesis (second gap, G2). DNA detection can be used to reflect the status of each phase of the cell cycle which is also named cell proliferation. DNA can bind to fluorescent dyes (such as propidium Iodide-PI), the fluorescent dyes binding to DNA at different stages are different, and the fluorescence intensity detected by flow cytometry can also be used to detect different phases in cell cycle.
When apoptotic cells occur, apoptotic bodies are produced due to the concentration and nucleolysis of cytoplasm and chromatin, which will change the light scattering properties of cells. In the early stage of apoptosis, the forward scattering (FSC) of cells decreased significantly, while the side scatter (SSC) increased or remained unchanged. In the late stage of apoptosis, both FSC and SSC signals decreased. Therefore, apoptotic cells can be observed by measuring the changes of light scattering by flow cytometry.
After staining with PI, assuming that the fluorescence intensity of G0/G1 phase cells is 1, the theoretical value of fluorescence intensity of G2/M phase cells containing double genomic DNA is 2, and the fluorescence intensity of S phase cells undergoing DNA replication is between 1 and 2. Apoptotic cells lost part of genomic DNA fragmentation due to nucleus concentration and DNA fragmentation. Therefore, apoptotic cells showed obvious weak staining after PI staining and the fluorescence intensity was less than 1. The sub-G1 peak appeared on the flow cytometry fluorescence map which is apoptotic cell.
The kit can be used to detect the DNA content (cell cycle) of cultured cells (suspension or adherence cells).

Reagent No Included

Absolute ethanol



Molt-4 cells were treated with 70% ethanol overnight and detected with this kit.

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Staining Procedure

1. Reagent Preparation

A. Store the absolute ethanol at -20 ℃ overnight.

B. Take out the RNase A reagent [E-CK-A351A] dissolve fully, mix it and put on ice for use.

2. Sample Preparation

A. Suspension Cells

1) Collect the cells, centrifuge at 300 g for 5 min and discard the supernatant. Add PBS to resuspend gently and count the cells.

2) Split the cell suspension into tubes, 5×10^5 cells for each. Centrifuge at 300 g for 5 min and discard the supernatant.

3) Add 0.3 mL PBS to resuspend the cells.

B. Adherent Cells

1) Adherent cells should be detached with trypsin and then collected sedimentary cells. Collect the cells, centrifuge at 300 g for 5 min and discard the supernatant. Add appropriate PBS to resuspend gently and count the cells.

2) Split the cell suspension into tubes, 5×10^5 cells for each. Centrifuge at 300 g for 5 min and discard the supernatant.

3) Add 0.3 mL PBS to resuspend the cells.

3. Take the resuspended cells to a tube with 1.2 mL absolute ethanol from -20 ℃ refrigerator, oscillate the cells and store at -20 ℃ for 1 h or overnight.

4. Centrifuge at 300 g for 5 min and discard the supernatant. Add 1 mL PBS to resuspend the cells, store at RT for 15 min.

5. Centrifuge at 300 g for 5 min and discard the supernatant. Add 100 μL RNase A reagent [E-CK-A351A] to resuspend the cells, incubate at 37 ℃ water bath for 30 min.

6. Add 400 μL Propidium Iodide (PI) Staining Solution [E-CK-A351B], mix fully and incubate at 2~8 ℃ for 30 min.

7. Analyze the cells immediately with proper machine settings.

Storage

RNase A Reagent [E-CK-A351A] should be stored at -20 ℃. Propidium Iodide (PI) Staining Solution [E-CK-A351B] should be stored at 2~8 ℃ in dark.

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Cautions

1. For maximal assay performance, this kit should be used within 12 months. Avoid freeze / thaw cycles.
2. The experimental results need to be detected by flow cytometry.
3. Detect apoptosis as soon as possible after staining to avoid increase in apoptosis or necrosis.
4. Avoid extended exposure of the samples to direct light to protect the fluorophores from quenching.
5. This kit is for research use only. For your safety and health, please wear lab clothes and gloves. Instructions should be followed strictly, changes of operation may result in unreliable results.

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