

## Cell Cycle Assay Kit (Blue Fluorescence)

Cat. No: E-CK-A353

Size: 20 Assays/50 Assays/100 Assays

Cat.	Products	20 Assays	50 Assays	100 Assays	Storage
E-CK-A351A	RNase A Reagent	1 mL×2	5 mL×1	10 mL×1	-20 °C
E-CK-A353A	Cycle Blue Reagent	8 mL×1	10 mL×2	10 mL×4	-20 °C, shading light
	Manual			One Copy	

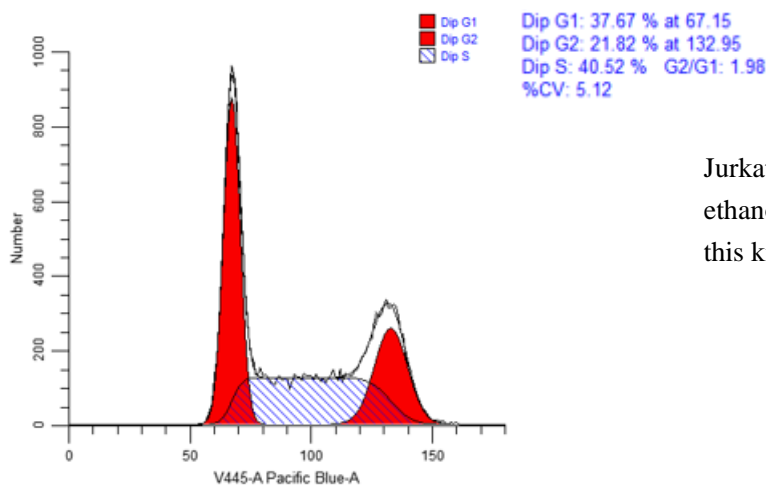
### Storage

RNase A Reagent should be stored at -20 °C for 1 year. Cycle Blue Reagent should be stored at -20 °C in dark for 1 year.

### Introduction

Elabscience® Cell Cycle Assay Kit (Fluorometric-Red) is a kit that detects cell cycle by detecting DNA content. 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 be divided into phases like interphase and Metaphase. Interphase 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 can bind to some specific fluorescent dyes (such as Cycle Blue), 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.

After staining with Cycle Blue, 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 Cycle Blue staining and the fluorescence intensity was less than 1. The sub-G1 peak appeared on the flow cytometry result which is apoptotic cell.



Jurkat cells were treated with 70% ethanol overnight and detected with this kit.

### For Research Use Only

## Staining Procedure

1. Reagent Preparation
  - A. Store the absolute ethanol at -20 °C overnight.
  - B. Take out the RNase A reagent dissolve fully, mix it and put on ice for use.
2. Sample Preparation
  - 1) Collect  $5 \times 10^5$  cells for each, centrifuge at  $300 \times g$  for 5 min and discard the supernatant.
  - 2) Add 1 mL PBS to resuspend gently. Centrifuge at  $300 \times g$  for 5 min and discard the supernatant.
  - 3) Add 0.3 mL PBS to resuspend the cells.
3. Add 0.7 mL absolute ethanol from -20 °C refrigerator, mix fully and store at -20 °C for 1 h or overnight.
4. Centrifuge at  $300 \times g$  for 5 min and discard the supernatant. Add 1 mL PBS to resuspend the cells, store at RT for 15 min.
5. Centrifuge at  $300 \times g$  for 5 min and discard the supernatant. Add 100  $\mu$ L RNase A reagent to resuspend the cells, incubate at 37 °C water bath for 30 min.
6. Add 400  $\mu$ L Cycle Blue Reagent, mix fully and incubate at 2-8 °C for 30 min in the dark.
7. Analyze the cells immediately with proper machine settings. The maximum excitation wavelength of Cycle Blue is 364 nm, and the maximum emission wavelength is 454 nm.

## Cautions

1. For maximal assay performance, this kit should be used within 12 months.
2. The experimental results need to be detected by flow cytometer.
3. Avoid extended exposure of the samples to direct light to protect the fluorophores from quenching.
4. Cycle Blue Reagent stimulates the human body. Please be careful during operation and pay attention to proper protection to avoid direct contact with the human body or inhalation.
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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