Cell Cycle Phase Determination Kit

Item No. 10009349

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Technical Support 888.526.5351
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GENERAL INFORMATION

Materials Supplied

Item Number	Item	100 Tests Quantity/Size	Storage
10009957	Propidium Iodide Reagent	1 vial/1 ml	4°C
10009322	Cell-Based Assay Buffer Tablets	2 Tablets	RT
10009959	RNase A Solution	1 vial/1 ml	-20°C
10009960	Cell Cycle Phase Determination Fixative	4 vials/25 ml	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A 6-, 12-, or 24-well plate for culturing cells
2. A flow cytometer with a 488 nm excitation laser
3. Distilled water

INTRODUCTION

Background

The progression of the cell cycle is controlled by a complex interplay of cell cycle regulators that either stimulate or inhibit the cell from entering each stage of the cell cycle. These molecules activate transcription factors which bind to DNA to turn on or off the production of proteins which result in cell division. Dysfunction of any step in this regulatory cascade causes abnormal cell proliferation, which underlies many human pathological conditions, most notably cancer and aging.[1] A crucial area in understanding these conditions is to understand the mechanisms underlying alterations in cell cycle progression.

Propidium iodide (PI) staining of DNA in permeabilized cells and its detection by flow cytometry is widely used to determine the percentage of cells in each phase of the cell cycle. Because PI intercalates into the base pairs of double-stranded DNA, the fluorescent intensity of a stained cell is directly proportional to the DNA content of the cell. Cells in G_0/G_1 are either resting or preparing to enter S (DNA synthesis) phase and contain one set of chromosomes. An exponentially growing population of cells displays a DNA content distribution containing two major peaks, a narrow peak on the left side representing G_0/G_1 phase cells and a second smaller peak on the right side representing G_2/M phase cells which contain two copies of all chromosomes. Cells in S phase are in the process of DNA replication; their DNA content distribution will fall between the tall peak of G_0/G_1 phase and the second peak of G_2/M phase. Since PI also binds to double-stranded RNA, RNase is included to degrade RNA that may interfere with the DNA content determination.

About This Assay

Cayman's Cell Cycle Phase Determination Kit provides a convenient tool for studying the induction and inhibition of cell cycle progression in any cell suspension sample. The assay involves the fixation and permeabilization of the cells of interest, making possible the staining of DNA within intact cells by propidium iodide. This kit will allow the investigator to determine the percentage of cells in a given sample that are in G_0/G_1, G_2, or S phase at the time of fixation, as well as to quantify cells in the sub-G_1 phase prior to apoptosis.

ASSAY PROTOCOL

Preparation of Assay-Specific Reagents

1. **Assay Buffer Preparation**

   Dissolve two cell-based assay buffer tablets (Item No. 10009322) in 200 ml of distilled water. This buffer should be stable for approximately one year at room temperature.

2. **Staining Solution Preparation**

   Immediately prior to staining fixed cell samples, prepare fresh staining solution as follows: for every 20 samples to be stained, mix 10 ml assay buffer with 200 µl RNase A solution (Item No. 10009959), and 200 µl PI reagent (Item No. 10009957).

Performing the Assay

1. Seed cells in a 6-, 12-, or 24-well plate at a density of 10^5-10^6 cells/well in 2, 1, or 0.5 ml of culture medium. Culture the cells in a CO_2 incubator at 37°C for at least 24 hours before treatment.

   *NOTE: At this point, it is recommended that the culture medium be changed to serum-free or low-serum medium to facilitate cycle synchronization. See **Troubleshooting** notes on page 10.*

2. Treat cells with or without experimental compounds. At the end of treatment, trypsinize (adherent cells), or collect cells (suspension cells). Centrifuge to pellet the cells, washing twice with assay buffer.

3. Resuspend the cell pellet to a density of 10^6 cells/ml in assay buffer. It is important to achieve a monodisperse cell suspension at this step by pipetting up and down repeatedly.

4. Add an equal volume of cell cycle phase determination fixative (Item No. 10009960) to each sample to fix and permeabilize the cells and place at -20°C for at least two hours. At this point, cells may be stored at -20°C for up to several months before staining with PI.

5. When ready to stain the cells with PI, centrifuge the fixed cells at 500 x g for five minutes. Decant fixative thoroughly.
6. Suspend the cell pellet in 0.5 ml staining solution. Incubate for 30 minutes at room temperature in the dark.
7. Analyze the samples on your flow cytometer using the following parameters:
   a. Set the PI channel (usually the 488 nm excitation laser, 650 nm emission filter) to linear, rather than logarithmic, acquisition.
   b. Acquire height as well as area in the PI channel.
   c. Adjust voltage to center the G_1 peak around the lower 30% of the PI histogram.
   d. Display PI area *versus* PI height as a dot plot to gate single cells, as shown in Figure 1A.
   e. Acquire at least 10,000 events per sample.

ANALYSIS

Performance Characteristics

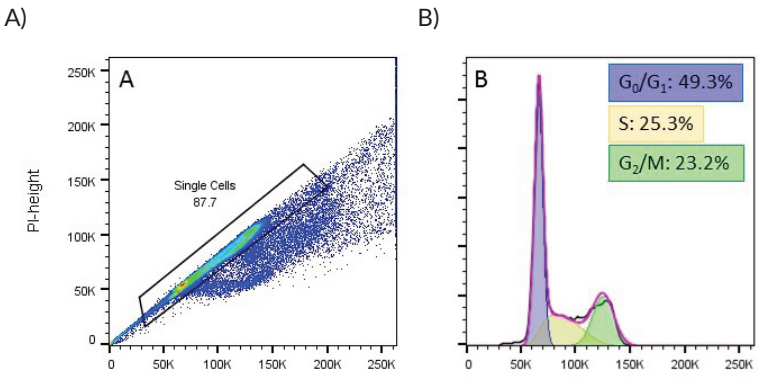

Figure 1: Sample flow cytometric gating analysis for cell cycle.
THP-1 cells were fixed and stained for cell cycle analysis according to the protocol in this kit booklet. Events were collected on a Miltenyi MACSQuant cytometer and data were analyzed in FlowJo by TreeStar, Inc. *A:* Doublet discrimination by gating cells in PI-area *versus* PI-height. *B:* Cell cycle analysis using the Dean-Jett-Fox model to calculate percentages of G_0/G_1, S, and G_2/Mi phase cells.

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Low sensitivity of the assay, cell cycle phases not well separated	Cells in each sample are not synchronized	It is recommended that cells in each sample be synchronized to the same cell cycle phase; this may be done by culturing the cells in serum-free or serum-depleted media for 24-48 hours prior to treatment, depending on cell type
Poor signal	The amount of staining solution is not optimal	The amount of staining solution needed to obtain a good cytometric reading may need to be adjusted, according to cell type

Reference

1. Malumbres, M. Cyclin-dependent kinases. *Genome Biol.* **15(6)**, 122 (2014).

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.