

7-AAD Cell Cycle Analysis Kit (with RNase)

| <u>Catalog Number</u> | <u>Size</u> |
|-----------------------|-------------|
| FXP0311-50 | 50Tests |
| FXP0311-100 | 100 Tests |
| FXP0311-200 | 200 Tests |



7-AAD Cell Cycle Analysis Kit (with RNase) provides a rapid and convenient assay for cell cycle and cell proliferation using flow cytometry. This package insert must be read in its entirety before using this product. If you have questions or experience problems with this product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

FOR RESEARCH USE ONLY NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTRODUCTION

For normal cells, the content of DNA is changed with the process of cell cycle. Observed DNA stained by dyes using flow cytometry to calculate percentage of G_0/G_1 , S, and G_2/M . It will be clear known that how about the distribution of cell cycle and the activity of proliferation. For apoptotic cells, DNAs in cells is degraded by endogenous nuclease activated and diffuse out of cells with the process of apoptosis.

A highly definable sub- G_1 peak occurs and is easily quantified by dyes. The change of DNA in apoptotic cells is also assayed for sorting and further analyzing apoptotic cells. After RNA is degraded by RNase, the nucleic acid dye in this kit bind with DNA composed of chromatin in the nucleus. And the results can be analyzed by flow cytometry.

REAGENTS

7-AAD Cell Cycle Analysis Kit (with RNase) is used for detecting the different stages of cell cycle.

Table 1: Reagents of the Kit

| Reagents | 50T | 100T | 200T |
|--------------------|-------|-------|-------|
| Staining buffer | 5ml | 10ml | 20ml |
| 7-AAD (25X) | 0.2ml | 0.4ml | 0.8ml |
| RNase A (2.5mg/ml) | 0.1ml | 0.2ml | 0.4ml |

KEY FEATURES

1. Easy to perform: simple and rapid procedure to perform.
2. Versatile: this kit is used for detecting cell cycle not only suspension cells but also adherence cells
3. Direct quantitation for normal, apoptotic, and dead cells by flow cytometry.
4. Ready to use
5. Highly competitive price

STORAGE

This kit remains stable for at least 18 months if stored at 4°C and protected from light.

THE REQUIRED ITEMS (not provided, but can help to buy):

1. 5 ml and 10 ml graduated pipettes
2. 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips
3. Beakers, flasks, cylinders necessary for preparation of reagents
4. Glass-distilled or deionized water, 95% ethanol and RNase
5. PBS (for 1 liter: 8.00g NaCl, 0.20 g KCl, Na₂HPO₄·12 H₂O 2.85 g, 0.20 g KH₂PO₄)
6. Bench top centrifuge

7. Flow Cytometer

PRECAUTIONS FOR USE

1. All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.
2. Do not mix or substitute reagents with those from other lots or other sources.
3. Do not use kit reagents beyond expiration date on label.
4. Do not expose kit reagents to strong light during storage or incubation.
5. Do not eat or smoke in areas where kit reagents or samples are handled.
6. Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
7. Avoid splashing or generation of aerosols.
8. In order to avoid microbial contamination or cross-contamination of reagents which may invalidate the test use disposable pipette tips and/or pipettes.
9. Glass-distilled water or deionized water must be used for reagent preparation.
10. Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
11. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

GENERAL PROTOCOL

1. Induce cell apoptosis using proper method and set a negative control. Harvest cells.
2. Add PBS to wash cells once. Then, centrifuge cells at 300g for five minutes.
3. Add PBS to resuspend cell and adjust cell concentration to 1×10^7 /ml.
4. Centrifuge cells at 300g for five minutes and discard the supernatant.

5. Fix cells using 70% ethanol at 4°C for two hours or overnight. Take 4 ml precooled 95% ethanol, add 1 ml cell suspension drop by drop into ethanol on low speed vortex oscillation , mix and fix at 4 ° C for 2 hours or more.
6. Use PBS to wash cells for removing fixing solution. If necessary, filter cell suspension once using sieve with 200 meshes.
7. Preparation of 7-AAD dyeing solution

| | 1 Sample | 6 Samples | 12 Samples |
|---------------------------|-----------------|------------------|-------------------|
| Staining buffer | 0.1ml | 0.6ml | 1.2ml |
| 7-AAD (25X) | 4μl | 24μl | 48μl |
| RNase A (2.5mg/ml) | 2μl | 12μl | 24μl |

8. Add 100 μl 7-AAD dyeing solution to cells suspension and incubate cells at 37 °C for 30 minutes.
9. Observe at of by flow cytometry. The maximum emission wavelength is 647 nm, and the excitation wavelength is 488 nm or 535nm.

RELATED PRODUCTS

Table 2: Related products

| Products name | Catalog number | size |
|---|-----------------------|---------------|
| PI Cell Cycle Analysis Kit | FXP021 | 50T/100T/200T |
| PI Cell Cycle Analysis Kit (with RNase) | FXP0211 | 50T/100T/200T |
| 7-AAD Cell Cycle Analysis Kit | FXP031 | 50T/100T/200T |
| 7-AAD Cell Cycle Analysis Kit (with RNAase) | FXP0311 | 50T/100T/200T |

If you have any questions, please tell us!