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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Elabscience®

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

Mitochondrial Membrane Potential Assay Kit (with JC-1)

Catalog No： E-CK-A301

Size： 20 Assays / 50 Assays / 100 Assays

Cat.	Products	20 Assays	50 Assays	100 Assays	Storage
E-CK-A301A	JC-1 Reagent	20 μL	50 μL	100 μL	-20°C
E-CK-A301B	JC- 1 Assay Buffer (10 ×)	4 mL	10 mL	10 mL× 2	2~8°C
E-CK-A301C	CCCP(10 mM)	40 μL	40 μL	40 μL	-20°C
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.

Introduction

Elabscience® Mitochondrial Membrane Potential Assay Kit (with JC-1) is developed to identify apoptotic with JC-1 reagent by changing of mitochondrial membrane potential which occur in early apoptotis.

This kit provides Carbonyl cyanide m-chlorophenylhydrazone (CCCP)[E-CK-A301C] to induce the decrease of mitochondrial membrane potential as a positive control reagent.

Detection principle

The decrease of mitochondrial membrane potential is a marker event in the early stage of apoptosis. It occurs before the appearance of nuclear apoptotic characteristics (chromatin concentration and DNA fragmentation). Once the mitochondrial membrane potential collapses, apoptosis is irreversible.

In normal cells, the mitochondrial membrane potential is high, JC-1 gathers in the matrix of mitochondria to form a polymer, which yields a red to orange colored emission (590±17.5 nm). In the early stage of apoptosis, the mitochondrial membrane potential decreases, JC-1 can't gather and it is a monomer which yields green fluorescence with emission of 530±15 nm.

Reagents not included

PBS, DI water.

Instructions

1×JC-1 Assay Buffer preparation

JC- 1 Assay Buffer (10 ×) [E-CK-A301B] is concentrated, diluted with DI water to 1 ×working solution before use. Store at 2~8℃.

For example: take 100 μL JC- 1 Assay Buffer (10 ×), add to 900 μL DI water, the mixture is 1× JC- 1 Assay Buffer.

JC-1 Staining Buffer preparation

1. Take 100 μL JC- 1 Assay Buffer (10 ×), add to 900 μL DI water, mix and heat to 37℃.
2. Add 2 μL JC-1 Reagent [E-CK-A301A] to the mixture. Mix until JC-1 Reagent fully dissolved. The mixture is JC-1 Staining Buffer.

Tip：JC-1 has a low solubility in water, it can be heated at 37℃ to promote dissolution.

Positive Control preparation

1. CCCP(10 mM)[E-CK-301C] dilute at 1:1000 to cell culture medium, and the CCCP is at 10 μM. Add the cell culture medium to cell and incubate for 20 min.
2. Follow the experiment procedure to detect the mitochondrial membrane potential.

Tips：For most cells, the mitochondrial membrane potential would be completely lost after 20 min of CCCP treatment at 10 μM and JC-1 stained cells showed green fluorescence, while normal cells showed red fluorescence after JC-1 staining.
For specific cells, the concentration and the incubation time of CCCP may be different, please refer to the relevant literature to determine.

Experimental Procedure

1. Deploy the 1×JC-1 Assay Buffer and JC-1 Staining Buffer according to the requirement of the experiment. See the instructions above for detail. The buffer should be stored at 2~8 ℃.
2. Set the positive Control. See the instructions above for detail. Induce apoptosis of suspension cells with reagents of interest.
3. Collect the cells, centrifuge at 300 g for 5 min and discard the supernatant. Add PBS to resuspend gently and count the cells.

Tips：Cell viability is the key to the experiment. When the adherent cells are used for apoptotic detection, treatments like digestion may increase the ratio of necrotic or apoptotic cells and cause uncontrollable effects on the experimental results. Please be aware!

4. Split the cell suspension into tubes, 1~5 $\times 10^5$ cells for each. Centrifuge at 300 g for 5 min and discard the supernatant. Add PBS to wash the cells and discard the supernatant.
5. Add 500 μL JC-1Staining Buffer to resuspend the cells. Incubate at 37 ℃, 5% CO_2 incubator for 15~20 min.

TIP: The incubation time is depending on cell types. In general, mammalian cells are recommended at 37 ℃. Other species should according to cell culture conditions.

6. Centrifuge at 300 g for 5 min and discard the supernatant. Add 500 μL pre-cold 1x JC-1 Assay Buffer to wash the cells twice.
7. Add 500 μL pre-cold 1x JC-1 Assay Buffer to resuspend the cells.
8. Analyze the cells immediately with proper machine settings. Otherwise, place the cells on ice in the dark and analyze within 0.5 h.

Storage

JC-1 Assay Buffer (10 ×) [E-CK-A301B] should be store at 2~8 ℃, other reagent should be stored at -20 ℃.

JC-1 Reagent [E-CK-A301A] should be stored in dark. Avoid freeze / thaw cycles.

Cautions

1. For maximal assay performance, this reagent should be used within 12 months. Avoid freeze / thaw cycles.
2. JC-1 Reagent may coagulate or precipitate at lower temperatures. Please heat at 20~25 ℃ water bath until it is completely dissolved.
3. IF the cells sensitive to pH changes, please use fetal bovine serum to replace JC-1 Assay Buffer for incubation or prolong observation time.
4. Avoid extended exposure of the samples to direct light to protect the fluorophores from quenching.
5. For your safety and health, please wear the lab coat and disposable gloves before the experiments.