线粒体膜电位检测试剂盒（JC-1）

产品组成：

产品名称	包装
JC-1(200×)	100μL/管，共 5 管
超纯水	90mL
JC-1 染色缓冲液(5×)	80mL
CCCP(10mM)	20μL

产品简介：

线粒体膜电位检测试剂盒（JC-1）是一种以 JC-1 为荧光探针，快速灵敏地检测细胞、组织或纯化的线粒体膜电位变化的试剂盒，可以用于早期的细胞凋亡检测。

JC-1 是一种广泛用于检测线粒体膜电位△Ψm 的理想荧光探针。可以检测细胞、组织或纯化的线粒体膜电位。在线粒体膜电位较高时，JC-1 聚集在线粒体的基质中，形成聚合物，可以产生红色荧光；在线粒体膜电位较低时，JC-1 不能聚集在线粒体的基质中，此时 JC-1 为单体，可以产生绿色荧光。这样就可以非常方便地通过荧光颜色的转变来检测线粒体膜电位的变化。常用红绿荧光的相对比例来衡量线粒体去极化的比例。

线粒体膜电位的下降是细胞凋亡早期的标志性事件。通过 JC-1 从红色荧光到绿色荧光的转变可以很容易地检测到细胞膜电位的下降，同时也可以用 JC-1 从红色荧光到绿色荧光的转变作为细胞凋亡早期的一个检测指标。

JC-1 单体的最大激发波长为 515nm，最大发射波长为 529nm；JC-1 聚合物的最大激发波长为 585nm，最大发射波长为 590nm。实际观察时，使用常规的观察红色荧光和绿色荧光的设置即可。

本试剂盒提供了 CCCP 作为诱导线粒体膜电位下降的阳性对照。

对于六孔板中的样品，本试剂盒共可以检测 100 个样品；对于 12 孔中的样品，本试剂盒共可检测 200 个样品。

使用说明：

JC-1 染色工作液的配制

六孔板每孔所需 JC-1 染色工作液的量为 1mL，其他培养器皿的 JC-1 染色工作液的用量以此类推：对于细胞悬液每 50～100 万细胞需 0.5mL JC-1 染色工作液。取适量 JC-1（200×），按照每 50μL JC-1（200×）加入 8mL 超纯水的比例稀释 JC-1。剧烈震荡充分溶解并混匀 JC-1。然后再加入 2mL JC-1 染色缓冲液（5×），混匀后即为 JC-1 染色工作液。

阳性对照的设置：

把试剂盒中提供的 CCCP（10mM）推荐按照 1：1000 的比例加入到细胞培养液中，稀释至 10μM，处理细胞 20 分钟。随后按照下述方法装载 JC-1，进行线粒体膜电位的检测。对于大多数细胞，通常 10μM CCCP 处理 20 分钟后线粒体的膜电位会完全丧失，JC-1 染色后观察应呈绿色荧光；而正常的细胞经 JC-1 染色后应显示红色荧光。对于特定的细胞，CCCP 的作用浓度和作用时间可能有所不同，需自行参考相关文献资料决定。

对于悬浮细胞

取 10 ~ 60 万细胞，重悬于 0.5mL 细胞培养液中，细胞培养液中可以含血清和酚红。

加入 0.5mL JC-1 染色工作液，颠倒数次混匀。细胞培养箱中 37℃孵育 20 分钟。

在孵育期间，按照每 1mL JC-1 染色缓冲液（5×）加入 4mL 蒸馏水的比例，配制适量的 JC-1 染色缓冲液(1×)，并放置于冰浴。

37℃孵育结束后，600g 4℃离心 3 ~ 4 分钟，沉淀细胞。弃上清，注意尽量不要吸除细胞。

用 JC-1 染色缓冲液（1×）洗涤 2 次：加入 1mL JC-1 染色缓冲液（1×）重悬细胞，600g 4℃离心 3 ~ 4 分钟，沉淀细胞，弃上清。再加入 1mL JC-1 染色缓冲液（1×）重悬细胞，600g 4℃离心 3 ~ 4 分钟，沉淀细胞，弃上清。

再用适量 JC-1 染色缓冲液（1×）重悬后，用荧光显微镜或激光共聚焦显微镜观察，也可以用荧光分光光度计检测或流式细胞仪分析。

对于贴壁细胞

注意：对于贴壁细胞，如果用荧光分光光度计或流式细胞仪检测，先收集细胞，重悬后参考悬浮细胞的检测方法。

对于六孔板的一个孔，吸除培养液，根据具体实验如有必要可以用 PBS 或其它适当溶液洗涤细胞一次，加入 1mL 细胞培养液。细胞培养液中可以含有血清和酚红。

加入 1mL JC-1 染色工作液，充分混匀。细胞培养箱中 37℃孵育 20 分钟。

在孵育期间，按照每 1mL JC-1 染色缓冲液（5×）加入 4mL 蒸馏水的比例，配制适量的 JC-1 染色缓冲液（1×），并放置于冰浴。

37℃孵育结束后， 吸除上清，用 JC-1 染色缓冲液（1×）洗涤 2 次。

加入 2mL 细胞培养液，培养液中可以含有血清和酚红。

荧光显微镜或激光共聚焦显微镜下观察。

对于纯化的线粒体

把配制好的 JC-1 染色工作液再用 JC-1 染色缓冲液（1×）稀释 5 倍。

0.9mL 5 倍稀释的 JC-1 染色工作液中加入 0.1mL 总蛋白量为 10 ~ 100μg 纯化的线粒体。

用荧光分光光度计或荧光酶标仪检测：混匀后直接用荧光分光光度计进行时间扫描（time scan），激发波长为 485nm，发射波长为 590nm。如果使用荧光酶标仪，激发波长不能设置为 485nm 时，可以在 475 ~ 520nm 范围内设置激发波长。另外，也可以参考下面步骤 6 中的波长设置进行荧光检测。

用荧光显微镜或激光共聚焦显微镜观察：方法同下面的步骤 6。

荧光观测和结果分析

检测 JC-1 单体时可以把激发光设置为 490nm ,发射光设置为 530nm ;检测 JC-1 聚合物时 ,可以把激发光设置为 525nm，发射光设置为 590nm。注意：此处测定荧光时不必把激发光和发射光设置在最大激发波长和最大发射波长。如使用荧光显微镜观察，检测 JC-1 单体时可以参考观察其它绿色荧光时的设置，如观察 GFP 或 FITC 时的设置；检测 JC-1 聚合物时可以参考观察其它红色荧光，如碘化丙啶或 Cy3 时的设置。出现绿色荧光说明线粒体膜电位下降，并且该细胞很可能处于细胞凋亡早期。出现红色荧光说明线粒体膜电位比较正常，细胞的状态也比较正常。

保存条件：

-20℃避光保存，尽量避免反复冻融，有效期一年。超纯水和 JC-1 染色缓冲液（5×）也可 4℃保存。

注意事项：

JC-1（200×）在 4℃、冰浴等较低温度情况下会凝固而粘在离心管管底、管壁或管盖内，可以 20 ~ 25℃水浴温育片刻至全部融解后使用。

必须先把 JC-1（200×）用试剂盒提供的超纯水充分溶解混匀后，才可以加入 JC-1 染色缓冲液（5×）。不可先配制 JC-1 染色缓冲液（1×）再加入 JC-1（200×），这样 JC-1 会很难充分溶解，会严重影响后续的检测。

装载完 JC-1 后用 JC-1 染色缓冲液（1×）洗涤时，使 JC-1 染色缓冲液（1×）保持 4℃左右，此时的洗涤效果较好。

JC-1 探针装载完并洗涤后尽量在 30 分钟内完成后续检测。在检测前需冰浴保存。

请勿把 JC-1 染色缓冲液（5×）全部配制成 JC-1 染色缓冲液（1×），本试剂盒使用过程中需直接使用 JC-1 染色缓冲液（5×）。

如果发现 JC-1 染色缓冲液（5×）中有沉淀，必须全部溶解后才能使用，为促进溶解可以在 37℃加热。

CCCP 为线粒体电子传递链抑制剂，有毒，请注意小心防护。

为了您的安全和健康，请穿实验服并戴一次性手套操作。

JC-1 Mitochondrial Membrane Potential Detection Kit.

Introduction

The loss of mitochondrial membrane potential ( ) is a hallmark for apoptosis. The JC-1 Assay Kit measures the mitochondrial membrane potential in cells. In non-apoptotic cells, JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) exists as a monomer in the cytosol (green) and also accumulates as aggregates in the mitochondria which stain red. Whereas, in apoptotic and necrotic cells, JC-1 exists in monomeric form and stains the cytosol green.

Background

The mitochondrial permeability transition is an important step in the induction of cellular apoptosis. During this process, the electrochemical gradient (referred to as) across the mitochondrial membrane collapses. The collapse is thought to occur through the formation of pores in the mitochondria by dimerized Bax or activated Bid, Bak, or Bad proteins. Activation of these pro-apoptotic proteins is accompanied by the release of cytochrome c into the cytoplasm (1-4). The JC-1 Assay Kit uses a unique cationic dye (5,5',6,6'-tetrachloro-1,1',3,3'tetraethylbenzimidazolylcarbocyanine iodide) to signal the loss of the mitochondrial membrane potential (5). In healthy cells, the dye stains the mitochondria bright red (6). The negative charge established by the intact mitochondrial membrane potential allows the lipophilic dye, bearing a delocalized positive charge, to enter the mitochondrial matrix where it accumulates. When the critical concentration is exceeded, J-aggregates form which become fluorescent red. In apoptotic cells, the mitochondrial membrane potential collapses, and the JC-1 cannot accumulate within the mitochondria. In these cells JC-1 remains in the cytoplasm in a green fluorescent monomeric form. Apoptotic cells, showing primarily green fluorescence, are easily differentiated from healthy cells which show red and green fluorescence. The aggregate red form has absorption/emission maxima of 585/590 nm (5). The green monomeric form has absorption/ emission maxima of 510/527 nm. Both apoptotic and healthy cells can be visualized simultaneously by fluorescence microscopy using a wide band-pass filter suitable for detection of fluorescein and rhodamine emission spectra. The JC-1 reagent is easy to use. Simply dilute the reagent in cell culture medium and add to the cells. After a 15 minute incubation, wash the cells and analyze by flow cytometry or fluorescence microscopy or fluorescence plate reader.

Warnings and Precautions

For Research Use Only. Not for use in diagnostic procedures.
Gloves, protective clothing and eyewear should be worn and safe laboratory practices followed.

Kit Components:

Reagents	Package
JC-1(200×) in DMSO	100μL/tube，5 tubes
Pure Water	90mL
Assay Buffer (5×)	80mL
CCCP(10mM)	20μL

Storage and Shelf Life:

Store the kit at 2°C to 8°C until first use. The performance of this product is guaranteed for 1 year from the date of purchase if stored and handled properly.
Reconstituted JC-1 reagent should be aliquoted in small amounts sufficient for one day of experimental work and stored at or below -20°C, protected from light and moisture (preferably in a desiccator). Shelf life of reconstituted JC-1 is six months if stored at or below -20°C.

Avoid multiple freeze-thaw cycles.

Preparation and Setup

Dilution of 5X Assay Buffer.

If necessary warm the 5X Assay Buffer until any salt crystals are completely dissolved.

Dilute the Assay Buffer 1:5 with pure water (e.g. 1ml 5X assay buffer + 4ml pure water).

Prepare JC-1 dye-loading solution

Thaw all the kit components at room temperature before use.

Add 25 L of 200X JC-1 into 5 mL of 1X Assay Buffer, and mix well.

Note: Aliquot and store the unused 200X JC-10 (Component A) at -20°C. Avoid repeated freeze/thaw cycles.

For Negative Control and Positive Control:

Treat cells with test compounds for a desired period of time to induce apoptosis. Set up parallel control experiments.

For Negative Control: Treat cells with vehicle only.

For Positive Control: Treat cells with 2uL CCCP in a 37°C, 5% CO2 incubator for 15 to 30 minutes.

Note: CCCP can be added simultaneously with JC-1 dye-loading solution. Titration of the CCCP may be required for optimal results with an individual cell lines.

A. Staining Protocol For Flow Cytometry

Cells should be cultured to a density not to exceed 1 x 10^6 cells/mL.

Each cell line should be evaluated on an individual basis to determine optimal cell density for apoptosis induction.

Induce apoptosis according to your specific protocol.

Transfer 0.5 mL cell suspension into a sterile centrifuge tube.

Centrifuge for 5 minutes at room temperature at 400 x g.

Remove the supernatant.

Resuspend cells in 0.5 ml 1X JC-1 Reagent solution prepared in step 4, above, under Dilution of JC-1 Reagent.

Incubate the cells at 37°C in a 5% CO_2 incubator for 15 minutes.

Centrifuge for 5 min at 400 x g and remove supernatant.

Resuspend the cell pellet in 2 mL cell culture medium or 1X Assay Buffer followed by centrifugation. Remove the supernatant.

Repeat step 9.

Resuspend the cell pellet in 0.5 mL fresh cell culture medim or 1X Assay Buffer. Cells are now ready for flow cytometry analysis.

Quantification by Flow Cytometry Analyze cells **immediately** following step 11 by flow cytometry. Mitochondria containing red JC-1 aggregates in healthy cells are detectable in the FL2 channel, and green JC-1 monomers in apoptotic cells are detectable in the FITC channel (FL1).

Instrument Setting up for Flow Cytometry

Two Parameter Analysis

Run the uninduced control sample first. Generate a log FL1 (X-axis) versus log FL2 (Y-axis) dot plot. Add regions R2 and R3 (as in Figure 2) to the dot plot.

Adjust the FL1and FL2 PMT voltages to register a dual positive population in region 2 (R2). The peak of the dual positive population should fall within the second and third log decade scale of FL1and FL2.

The region 2 (R2) gate should be adjusted to include >95% of events. This number will vary depending on the condition of the cells.

Run the induced sample, using the PMT settings established above for the uninduced control sample. One should

see a population of cells that appears in the region 3 (R3). This reflects a loss of red emission on the FL2 axis, which corresponds to the loss of mitochondrial membrane potential in induced cells.

If the induced sample exhibits only a minimal decrease in red emission, increase the FL2-%FL1 compensation.

Repeat steps 3 and 4. See Section 3: TECH NOTE #1: Flow Cytometer Settings See Section 3: TECH NOTE #2: Quadrant

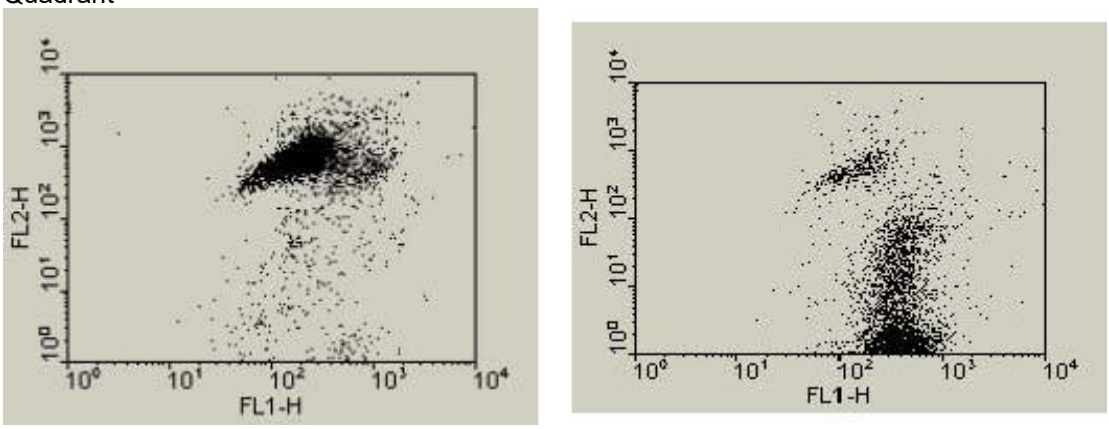

Figure 1. Mitochondrial Staining in Jurkat Cells Using JC-1. Flow Cytometry (Two Parameter Analysis)

Jurkat cells were treated with DMSO (Left) or 1.5 uM staurosporine (Right) for 3 hours. Cells were labeled with JC-1 reagent for 15 minutes. After washing, cells were analyzed on a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer. A dot plot of red fluorescence (FL2) versus green fluorescence (FL1) resolved live cells with intact mitochondrial membrane potential (Left) from apoptotic and dead cells with lost mitochondrial membrane potential (Right). Note the increase in cells numbers with decreased red fluorescence (Right).

B. Staining Protocol for Fluorescence Microscopy

1. Staining of Cells in Suspension

Cells should be cultured to a density not to exceed 1 x 10^6 cells/mL.

Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

Induce apoptosis according to your specific protocol.

Transfer 0.5 ml cell suspension into a sterile centrifuge tube.

Centrifuge for 5 minutes at room temperature at 400 x g.

Remove the supernatant.

Resuspend cells in 0.5 mL 1X JC-1 reagent prepared in step 4: Dilution of JC-1 Reagent

Incubate the cells at 37°C in a 5% CO_2 incubator for 15 minutes.

Centrifuge for 5 min at 400 x g and remove supernatant.

Resuspend the cell pellet in 2 mL 1X Assay Buffer followed by centrifugation. Remove supernatant.

Resuspend the cell pellet in 0.3 mL Assay Buffer.

Observe immediately with a fluorescence microscope using a “dual-bandpass” filter designed to simultaneously detect fluorescein and rhodamine or fluorescein and Texas Red*. In live non-apoptotic cells, the mitochondria will appear red following aggregation of the JC-1 reagent. The red aggregates emit at 590 nm. In apoptotic and dead cells, the dye will remain in its monomeric form and will appear green with an emission at 530 nm See Section 3: TECH NOTE #3: Fluorescence Filters

2. Staining of Monolayer Cells

Grow cells on a glass cover slip in a petri dish or in a chamberslide. Induce cells according to your specific protocol.

Dilute JC-1 reagent to 1X immediately prior to use. As in step 4 under Dilution of JC-1 Reagent

Note: To remove undissolved particles, centrifuge the dye/media solution for 1 minute at 13,000 x g and carefully transfer the supernatant without disturbing pelleted debris into a fresh tube.

Remove the cell culture media and replace with enough diluted 1X JC-1 reagent sufficient to cover the cells.

Incubate the cells at 37°C in a 5% CO_2 incubator for 15 minutes.

Remove media and wash once with 1X Assay Buffer.

Add a drop of PBS and cover with a coverslip.

Observe immediately with a fluorescence microscope using a “dual-bandpass” filter designed to simultaneously detect fluorescein and rhodamine or fluorescein and Texas Red. In live non-apoptotic cells, the mitochondria will appear red following aggregation of the JC-1 reagent. The red aggregates emit at 590 nm. In apoptotic and dead cells the dye will remain in its monomeric form and will appear green with an emission at 530 nm.

C. Staining Protocol for Fluorescence Ratio Detection

Cells should be cultured to a density not to exceed 1 x 106 cells/mL.

Each cell line should be evaluated on an individual basis to determine the optimal cell density for apoptosis induction.

Induce apoptosis according to your specific protocol.

Transfer 0.5 mL cell suspension into a sterile centrifuge tube.

Centrifuge for 5 minutes at room temperature at 400 x g.

Remove the supernatant.

Resuspend cells in 0.5 mL 1X JC-1 reagent prepared in step 4 under: Dilution of JC-1 Reagent

Incubate the cells at 37°C in a 5% CO2 incubator for 15 minutes.

Centrifuge for 5 min at 400 x g and remove supernatant.

Resuspend the cell pellet in 2 mL 1X Assay Buffer followed by centrifugation. Remove supernatant.

Repeat step 9.

Resuspend the cell pellet in 300 uL Assay Buffer.

Transfer 100 uL cell suspension into each of three wells of a black 96-well plate.

Measure red fluorescence (excitation 550 nm, emission 600 nm) and green fluorescence (excitation 485 nm, emission 535 nm) using a fluorescence plate reader.

Determine the ratio of red fluorescence divided by green fluorescence.

The ratio of red to green fluorescence is decreased in dead cells and in cells undergoing apoptosis compared to healthy cells.

Tech Notes

TECH NOTE #1: *Flow Cytometer Settings.* A typical setting for the analysis of JC-1 staining on a FACSCalibur (Becton Dickinson, San Jose, CA) flow cytometer is as follows: FL1 PMT voltage 511 FL2 PMT voltage 389 Compensation: FL1 – 10.5% FL2 FL2 – 25.9% FL1 TECH NOTE #2: *Quadrants*. On instruments where it is not possible to add regions to the dot plot, quadrants are added instead, using the following protocol:

Run the uninduced control sample first. Generate a log FL1 (X-axis) versus log FL2 (Y-axis) dot plot. Add quadrants to the dot plot.

Adjust the FL1and FL2 PMT voltages to register a dual positive population in quadrant 2 with the dual positive population falling within the second and third log decade scale of FL1and FL2.

The quadrant 2 markers should be adjusted so that the statistics read >95% on gated events.

Run the induced sample, using the PMT settings established above for the uninduced control sample. One should see a population of cells that appears in the 4th quadrant. This reflects a loss of red emission on the FL2 axis.

If the induced sample exhibits only a minimal decrease in red emission, increase the FL2-%FL1 compensation.

Repeat steps 3 and 4. TECH NOTE #3: Fluorescence Filters for Fluorescence Microscopy.

a. Both the red JC-1 aggregate and the green monomer can be viewed with a “double-bandpass” filter designed to simultaneously detect fluorescein and rhodamine or fluorescein and Texas RedTM*.

b. JC-1 aggregates can be viewed with a bandpass filter designed to detect rhodamine (excitation 540 nm, emission 570 nm) or Texas Red (excitation 590 nm, emission 610 nm).

c. JC-1 monomers are dected with a bandpass filter used for the detection of fluorescein (excitation 490 nm, emission 520 nm).

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