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线粒体膜电位检测试剂盒(JC-1)

产品编号	产品名称	包装
C2006	线粒体膜电位检测试剂盒(JC-1)	>100次

产品简介：

- 线粒体膜电位检测试剂盒(JC-1) (Mitochondrial membrane potential assay kit with 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(mitochondrial membrane potential) ΔΨm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(matrix)中，形成聚合物(J-aggregates)，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(monomer)，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一个标志性事件。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514nm，最大发射波长为529nm；JC-1聚合物(J-aggregates)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，本试剂盒共可以检测200个样品。

包装清单：

产品编号	产品名称	包装
C2006-1	JC-1(200X)	100μl/管，共5管
C2006-2	超纯水	90ml
C2006-3	JC-1染色缓冲液(5X)	80ml
C2006-4	CCCP(10mM)	20μl
—	说明书	1份

保存条件：

-20ºC保存。JC-1(200X)需避光保存，并尽量避免反复冻融。超纯水和JC-1染色缓冲液(5X)也可4ºC保存。

注意事项：

- JC-1(200X)在4ºC、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20-25ºC水浴温育片刻至全部融解后使用。
- 必须先把JC-1(200X)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(5X)。不可先配制JC-1染色缓冲液(1X)再加入JC-1(200X)，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(1X)洗涤时，使JC-1染色缓冲液(1X)保持4ºC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(5X)全部配制成JC-1染色缓冲液(1X)，本试剂盒使用过程中需直接使用JC-1染色缓冲液(5X)。
- 如果发现JC-1染色缓冲液(5X)中有沉淀，必须全部溶解后才能使用，为促进溶解可以在37ºC加热。
- CCCP为线粒体电子传递链抑制剂，对人体有害，操作时请小心，并注意有效防护以避免直接接触人体或吸入体内。
- 本产品仅限于专业人员的科学研究用，不得用于临床诊断或治疗，不得用于食品或药品，不得存放于普通住宅内。
- 为了您的安全和健康，请穿实验服并戴一次性手套操作。

使用说明：

1. 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，其它培养器皿的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(200X)，按照每50μl JC-1(200X)加入8ml超纯水的比例稀释JC-1。剧烈Vortex充分溶解并混匀JC-1。然后再加入2ml JC-1染色缓冲液(5X)，混匀后即为JC-1染色工作液。

2. 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确定。

3. 对于悬浮细胞：

a. 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。
b. 加入0.5ml JC-1染色工作液，颠倒数次混匀。细胞培养箱中37ºC孵育20分钟。
c. 在孵育期间，按照每1ml JC-1染色缓冲液(5X)加入4ml蒸馏水的比例，配制适量的JC-1染色缓冲液(1X)，并放置于冰浴。
d. 37ºC孵育结束后， 600g 4ºC离心3-4分钟，沉淀细胞。弃上清，注意尽量不要吸除细胞。
e. 用JC-1染色缓冲液(1X)洗涤2次：加入1ml JC-1染色缓冲液(1X)重悬细胞，600g 4ºC离心3-4分钟，沉淀细胞，弃上清。再加入1ml JC-1染色缓冲液(1X)重悬细胞，600g 4ºC离心3-4分钟，沉淀细胞，弃上清。
f. 再用适量JC-1染色缓冲液(1X)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。

4. 对于贴壁细胞：

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

a. 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。
b. 加入1ml JC-1染色工作液，充分混匀。细胞培养箱中37ºC孵育20分钟。
c. 在孵育期间，按照每1ml JC-1染色缓冲液(5X)加入4ml蒸馏水的比例，配制适量的JC-1染色缓冲液(1X)，并放置于冰浴。
d. 37ºC孵育结束后， 吸除上清，用JC-1染色缓冲液(1X)洗涤2次。
e. 加入2ml细胞培养液，培养液中可以含有血清和酚红。
f. 荧光显微镜或激光共聚焦显微镜下观察。

5. 对于纯化的线粒体：

a. 把配制好的JC-1染色工作液再用JC-1染色缓冲液(1X)稀释5倍。
b. 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。
c. 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。
d. 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。

6. 荧光观测和结果分析：

a. 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。

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