Annexin V-FITC细胞凋亡检测试剂盒
(TMR红色荧光)

产品编号

mla69980

产品描述

Annexin V-FITC 细胞凋亡检测试剂盒(Annexin V-FITC Apoptosis Detection Kit)是用 FITC 标记的重组人 Annexin V 来检测细胞凋亡时出现在细胞膜表面的磷脂酰丝氨酸的一种细胞凋亡检测试剂盒。可以使用流式细胞仪、荧光显微镜或其它荧光检测设备进行检测。Annexin V 选择性结合磷脂酰丝氨酸(phosphatidylserine，简称 PS)。磷脂酰丝氨酸主要分布在细胞膜内侧，即与细胞浆相邻的一侧。在细胞发生凋亡的早期，不同类型的细胞都会把磷脂酰丝氨酸外翻到细胞表面，即细胞膜外侧。磷脂酰丝氨酸暴露到细胞表面后会促进凝血和炎症反应。而 Annexin V 和外翻到细胞表面的磷脂酰丝氨酸结合后可以阻断磷脂酰丝氨酸的促凝血和促炎症反应活性。

用带有绿色荧光的荧光探针 FITC 标记的 Annexin V，即 Annexin V-FITC，就可以用流式细胞仪或荧光显微镜非常简单而直接地检测到磷脂酰丝氨酸的外翻这一细胞凋亡的重要特征。

本试剂盒还提供了碘化丙啶(Propidium Iodide, PI)染色液，碘化丙啶可以染色坏死细胞或凋亡晚期丧失细胞膜完整性的细胞，呈现红色荧光。对于坏死细胞，由于细胞膜的完整性已经丧失，Annexin V-FITC 可以进入到细胞浆内，与位于细胞膜内侧的磷脂酰丝氨酸结合，

从而也使坏死细胞呈现绿色荧光。

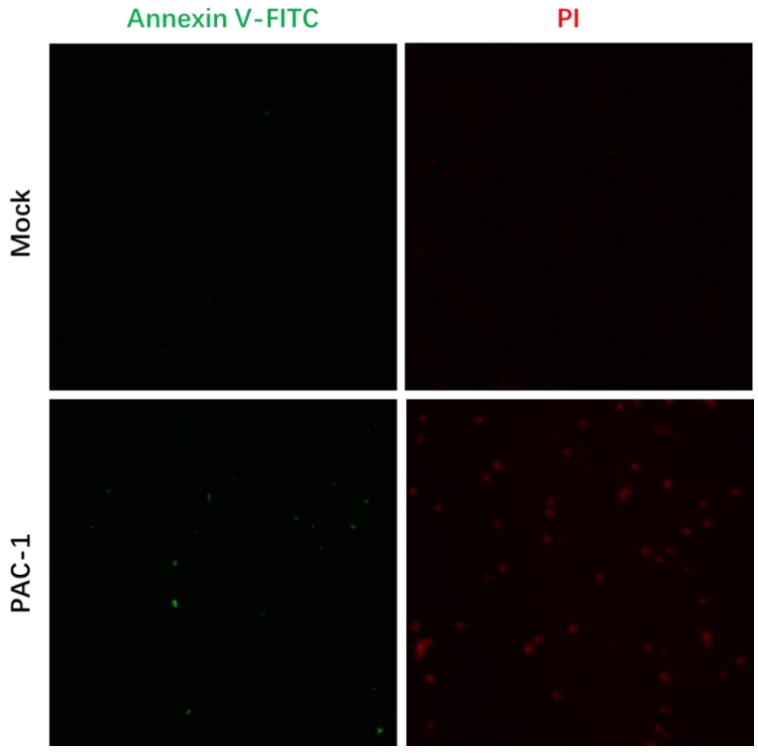
储存与运输

本试剂盒储存在-20℃，TMR-5-dUTP Labeling Mix 需避光储存于-20℃，有效期 12 个月。

组成

产品名称	产品包装
Annexin V-FITC	20μL
Annexin V-FITC 工作液	5μL
碘化丙啶染色液	100μL
说明书一份	

本试剂盒的荧光检测效果如下（PAC-1 作为凋亡诱导剂，作用于人肺癌细胞 NCIH292 1h）：



1.对于悬浮细胞：

A. 在进行完细胞凋亡刺激后，1000g(2000rpm)离心 5 分钟，弃上清，收集细胞，用 PBS 轻轻重悬细胞并计数。注意：PBS 重悬不能省略，PBS 重悬的过程同时也起到了洗涤细胞的作用，可以保证后续 Annexin V-FITC 的结合。

B. 取 5-10 万重悬的细胞，1000g 离心 5 分钟，弃上清，加入 199μl Annexin V-FITC 工作液轻轻重悬细胞。

C. 加入 1μl Annexin V-FITC，轻轻混匀。

D. 加入 5μl 碘化丙啶染色液，轻轻混匀。

E. 室温(20-25℃)避光孵育 20 分钟，随后置于冰浴中。

F. 随即进行流式细胞仪检测，Annexin V-FITC 为绿色荧光，碘化丙啶(PI)为红色荧光。如果用于荧光显微镜检测，1000g 离心 5 分钟，收集细胞，用 50-100μl Annexin V-FITC 结合液轻轻重悬细胞，涂片后，荧光显微镜下观察。

2.对于贴壁细胞的流式细胞术检测：

A. 将细胞培养液吸出至一合适离心管内，PBS 洗涤贴壁细胞一次，加入适量胰酶细胞消化液(无 EDTA)消化细胞。室温孵育至轻轻吹打可以使贴壁细胞吹打下来时，吸除胰酶细胞消化液。需避免胰酶的过度消化。

B. 加入步骤 2A 中收集的细胞培养液，稍混匀，转移到离心管内，1000g 离心 5 分钟，弃上清，收集细胞，用 PBS 轻轻重悬细胞并计数。注意：加入步骤 2A 中的细胞培养液一方面可以收集已经悬浮的发生凋亡或坏死的细胞，另一方面细胞培养液中的血清可以有效中和残留的胰酶；残留的胰酶会消化并降解后续加入的 Annexin V-FITC 导致染色失败。

C. 取 5-10 万重悬的细胞，1000g 离心 5 分钟，弃上清，加入 199μl Annexin V-FITC 工作液轻轻重悬细胞。

D. 加入 1μl Annexin V-FITC，轻轻混匀。

E. 加入 5μl 碘化丙啶染色液，轻轻混匀。

F. 室温(20-25℃)避光孵育 20 分钟，随后置于冰浴中。

G. 随即进行流式细胞仪检测，Annexin V-FITC 为绿色荧光，碘化丙啶(PI)为红色荧光。

3. 对于贴壁细胞的原位荧光显微镜检测：

A. 在凋亡诱导结束后，吸除细胞培养液，加入 PBS 洗涤一次。

B. 加入 199μl Annexin V-FITC 工作液。

C. 加入 1μl Annexin V-FITC，轻轻混匀。

D. 加入 5μl 碘化丙啶染色液，轻轻混匀。

E. 室温(20-25℃)避光孵育 20 分钟，随后置于冰浴中。

F. 随即在荧光显微镜下观察，Annexin V-FITC 为绿色荧光，碘化丙啶(PI)为红色荧光。

注：

1. ETDA 作为金属离子螯合剂，可以螯合 Ca2+,影响检测结果，请使用无 EDTA 的胰酶消化细胞。

2. 如果有细菌或真菌污染，会严重影响检测效果。

3. 染色后请在 30 分钟内完成检测，否则凋亡和坏死细胞数量会显著增加。

4. 荧光物质均易发生淬灭，在进行荧光观察时，尽量缩短观察时间，同时在操作和存放过程中也尽量注意避光保存。

5. 清洁细胞所使用的 PBS 及诱导凋亡的阳性刺激物需自备。

6. 实验过程中请穿实验服并戴一次性手套，口罩，避免污染，确保安全。

Annexin V-FITC/PI Apoptosis Detection Kit Instructions

1. For suspension cells

A. After inducing apoptosis, centrifuge the cells at 1000g (approximately 2000 rpm) for 5 minutes. Discard the supernatant, collect the cells, resuspend them gently in PBS and count the cell number. Note: Resuspension in PBS is indispensable, as it also serves the purpose of washing the cells to ensure the subsequent binding of Annexin V-FITC.

B. Take 50,000－100,000 resuspended cells, centrifuge at 1000g for 5 minutes, discard the supernatant, and add 199 μl of Annexin V-FITC working buffer to resuspend the cells gently.

C. Add 1 μl of Annexin V-FITC and mix gently.

D. Add 5 μl of propidium iodide (PI) staining solution and mix gently.

E. Incubate the mixture at room temperature (20－25°C) in the dark for 10－20 minutes, then place it on ice. Light shielding can be achieved using aluminum foil. During incubation, resuspend the cells 2–3 times to improve the staining effect.

F. Immediately perform flow cytometry analysis. Annexin V-FITC emits green fluorescence, while propidium iodide (PI) emits red fluorescence. If fluorescence microscopy is used for detection, centrifuge the cells at 1000g for 5 minutes, collect the cells, resuspend them gently in 50－100 μl of Annexin V-FITC binding buffer,

prepare cell smears, and observe under a fluorescence microscope.

2. For flow cytometry analysis of adherent cells

A. Aspirate the cell culture medium into an appropriate centrifuge tube. Wash the adherent cells once with PBS, add an appropriate amount of trypsin digestion solution (without EDTA) to digest the cells. Incubate at room temperature until the adherent cells can be detached by gentle pipetting, then aspirate the trypsin digestion solution. Avoid over-digestion with trypsin.

B. Add the cell culture medium collected in step 2A, mix slightly, transfer to a centrifuge tube, centrifuge at 1000g for 5 minutes, discard the supernatant, collect the cells, resuspend them gently in PBS and count the cell number. Note: Adding the cell culture medium collected in step 2A not only allows the collection of suspended apoptotic or necrotic cells, but also the serum in the medium can effectively inhibit or neutralize residual trypsin; residual trypsin will digest and degrade the subsequently added Annexin V-FITC, leading to staining failure.

C. Take 50,000－100,000 resuspended cells, centrifuge at 1000g for 5 minutes, discard the supernatant, and add 199 μl of Annexin V-FITC working buffer to resuspend the cells gently.

D. Add 1 μl of Annexin V-FITC and mix gently.

E. Add 5 μl of propidium iodide (PI) staining solution and mix gently.

F. Incubate the mixture at room temperature (20－25 °C) in the dark for 10－20 minutes, then place it on ice. Light shielding can be achieved using aluminum foil.

During incubation, resuspend the cells 2–3 times to improve the staining effect.

G. Immediately perform flow cytometry analysis. Annexin V-FITC emits green fluorescence, while propidium iodide (PI) emits red fluorescence.

3. For in situ fluorescence microscopy analysis of adherent cells

A. After the completion of apoptosis induction, aspirate the cell culture medium and wash the cells once with PBS.

B. Add 199 μl of Annexin V-FITC working buffer.

C. Add 1 μl of Annexin V-FITC and mix gently.

D. Add 5 μl of propidium iodide (PI) staining solution and mix gently.

E. Incubate the cells at room temperature (20–25°C) in the dark for 20 minutes, then place them on ice.

F. Immediately observe the cells under a fluorescence microscope. Annexin V-FITC emits green fluorescence, while propidium iodide (PI) emits red fluorescence.

Note:

1. As a metal ion chelator, EDTA can chelate Ca^{2+} and interfere with the test results; please use EDTA-free trypsin for cell digestion.

2. Bacterial or fungal contamination will seriously affect the test performance.

3. Please complete the test within 30 minutes after staining, otherwise the number of apoptotic and necrotic cells will increase significantly.

4. Fluorescent substances are prone to quenching. When conducting fluorescence observation, minimize the observation time as much as possible, and also keep

them away from light during operation and storage.

5. The PBS used for cell washing and the positive stimulant for inducing apoptosis need to be prepared by yourself.

6. Please wear a lab coat , disposable gloves and surgical mask during the experiment to avoid contamination and ensure safety.