

Annexin V-PE/7-AAD Apoptosis Detection Kit



Sino Biological
Biological Solution Specialist

Catalog Number: **APK10448-P**

Size: 20 Tests
100 Tests

Product Information

Application	Flow Cytometry
Storage and stability	Stored at 2°C - 8°C. Protected from prolonged exposure to light. Do not freeze ! All reagents are stable for one year from date of receipt under proper storage conditions.
Safety Caution	7-AAD is a potential carcinogen. Wearing protective clothing, gloves, and eye/face protection is recommended in order to avoid contact with skin and eyes.

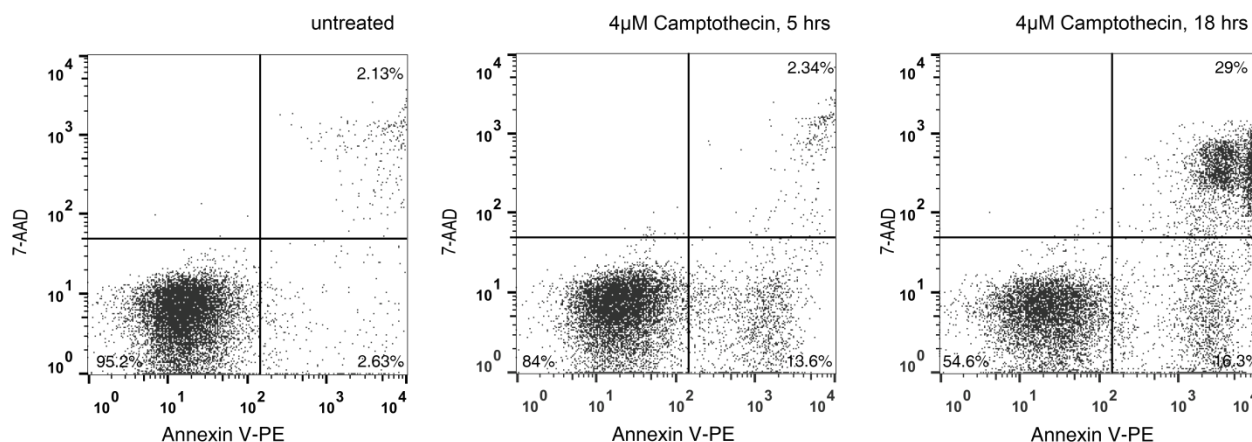
Components

Description	Size (20 Tests)	Size (100 Tests)	Vol. per Test	Formulation
Annexin V-PE (Cat: 10448-HNAE-P)	0.1 mL	0.5 mL	5 µL	Aqueous solution containing 0.5% BSA and 0.03% Proclin300
7-AAD	0.1 mL	0.5 mL	5 µL	Aqueous solution containing 0.5% BSA and 0.03% Proclin300
10× Binding Buffer	10 mL	50 mL	Please read the Staining Procedure	Aqueous buffered solution containing no preservative

Applications Tested

The Annexin V-PE/7-AAD Apoptosis Detection Kit has been tested on Jurkat cells treated with Camptothecin. Annexin V binding is calcium dependent and defined calcium and salt concentrations are required.

Investigators should note that the Annexin V-PE/7-AAD Apoptosis Detection Kit has not been routinely tested on adherent cell types. During cell detachment and harvesting, membrane damage may occur, and then bring about a false positive result. If an adherent cell type was used, it is recommended to pre-test the cell dissociation method. And it is best to avoid using cell detach solution with EDTA.



Flow Cytometric Analysis of Annexin V-PE staining. Jurkat cells were untreated (left panels), treated for 5 hours (middle panels) or 18 hours (right panels) with 4µM Camptothecin (Sigma, Cat.No C9911). Cells were incubated with Annexin V-PE and 7-AAD and analyzed by flow cytometry. Untreated cells were primarily Annexin V-PE and 7-AAD negative. After 5 hours treatment, there were primarily two populations of the cells: cells that were viable and not undergoing apoptosis (Annexin V-PE and 7-AAD negative); cells undergoing apoptosis (Annexin V-PE positive and 7-AAD negative). A minor population of cells were observed to be Annexin V-PE and 7-AAD positive, they were in end stage apoptosis or already dead. After 18 hours treatment, the proportion of the cells at the end stage of apoptosis or dead ones (Annexin V-PE and 7-AAD positive) was significantly increased.

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Staining Procedure

➤ *Please quick-spin vial before opening, for maximal recovery of contents.*

1. Wash cells twice with cold PBS, and then resuspend cells in $1 \times$ Binding Buffer at a concentration of $0.1 \times 10^7 \sim 1 \times 10^7$ cells/mL.
 $1 \times$ Binding Buffer : Dilute 1 part of the $10 \times$ Binding Buffer to 9 parts of distilled water.
2. Transfer 100 μ L of cell suspension to a tube.
3. Add 5 μ L Annexin V-PE and 5 μ L 7-AAD, gently mix the cells and incubate for 15 min at room temperature (25°C) in the dark.
4. Add 400 μ L of 1X Binding Buffer to each tube. Analyze by flow cytometry within 1 hr.

Notes

1. For accurate results, suggested the following controls to set up flow cytometry.
 - a) Negative control: Unstained cells.
 - b) Single color control: Cells stained with Annexin V-PE (no 7-AAD). Cells stained with 7-AAD (no Annexin V-PE).

The negative control and single color control are used to set up compensation and quadrants.

- c) Experimental control: the untreated cell population, used to define the basal level of apoptotic and dead cells.
 - d) Optional control: To demonstrated the specific of Annexin V-PE, cells incubated with purified recombinant Annexin V (10448-HNAE) to block Annexin V-PE binding sites prior to adding Annexin V-PE.
2. Do not use after expiration date.
 3. Avoided to mix the different batches.

Background

Apoptosis is a process of programmed cell death that occurs in multicellular organisms. It is a programmed cell death mechanism characterized by loss of plasma membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, chromosomal DNA fragmentation, and global mRNA decay. One of the earliest features is the change of plasma membrane.

Annexin V, also known as Annexin A5 (ANXA5), they are abundant intracellular proteins, and several different annexin gene products are expressed in all mammalian cells examined to date. Annexin V belongs to a family of Ca^{2+} binding proteins that undergo reversible Ca^{2+} -dependent binding to phospholipids (PLs). In healthy cells, phosphatidylserine (PS) is predominantly located along the cytosolic side of the plasma membrane, upon initiation of apoptosis, the PS is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment, Annexin V has a high affinity for PS, and binds to cells with exposed PS. Annexin V may be conjugated to fluorochromes including FITC, PE. This format retains its high affinity for PS and thus serves as a sensitive probe for flow cytometric analysis of cells that are undergoing apoptosis.

7-AAD (7-amino-actinomycin D) has a high DNA binding constant and is efficiently excluded by intact cells, can be used in place of propidium iodide (PI) for the exclusion of nonviable cells. Different from PI, the 7-AAD has minimal spectral overlap with phycoerythrin (PE) and fluorescein isothiocyanate (FITC), and can be used in conjunction with PE and FITC-labelled antibodies in multicolor analysis.

In early stage apoptosis, the PS was exposed on the cell surface, but the plasma membrane excludes 7-AAD. These cells will stain with Annexin V but not 7-AAD, thus distinguishing cells in early apoptosis (Annexin V positive, 7-AAD negative). In late stage apoptosis, the cell membrane loses integrity thereby allowing 7-AAD access and bind to the DNA, at the same time allowing Annexin V access binding with the PS in the interior of the cell (Annexin V positive, 7-AAD positive). However this assay can't distinguish the cells that died undergone apoptotic with those that have died as a result of necrosis, in either case, the dead cells will stain with both Annexin V and 7-AAD.

	Annexin V	7-AAD
Viable cells	—	—
Early apoptotic cells	+	—
Late apoptotic or dead cells	+	+

+: Positive
—: Negative

Reference

1. Cederholm A. et al., 2007, Ann N Y Acad Sci. 1108: 96-103.
2. Schlaepfer DD. et al., 1992, Biochemistry. 31:1886-91.
3. Vermes I. et al., 1995, J Immunol Methods. 184 (1): 39–51.

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Annexin V-PE/7-AAD 凋亡检测试剂盒

货号：APK10448-P

Size: 20 Tests
100 Tests



产品信息

应用	流式
储存及稳定性	2℃-8℃避光保存，勿冷冻。 本试剂盒所有组份依保存条件储存，自收到之日起一年有效。
安全注意事项	7-AAD是一种潜在的致癌物。为了您的安全和健康，操作时请穿实验服、戴手套、做好眼睛及面部防护，避免7-AAD接触皮肤及眼睛。

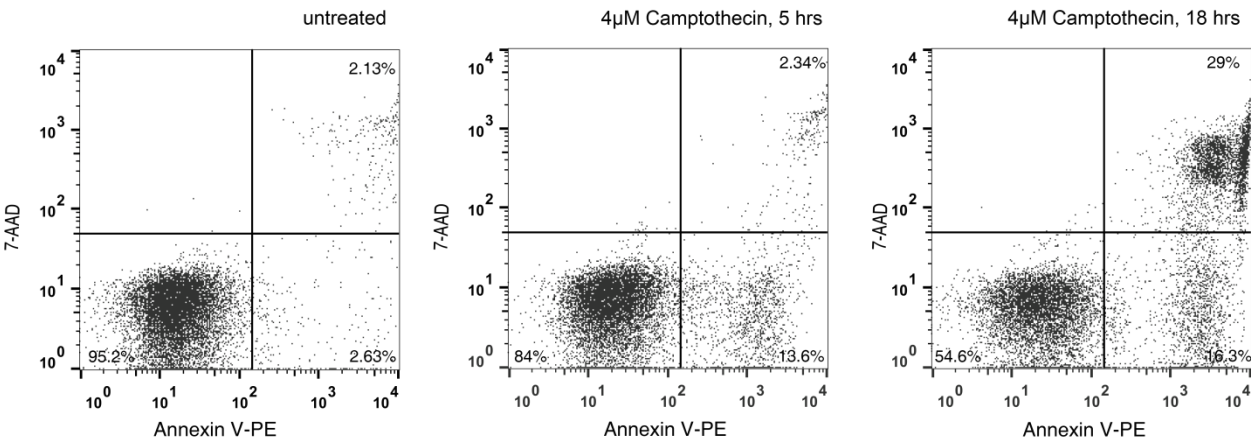
组份

名称	规格 (20 Tests)	规格 (100 Tests)	每Test使用量	缓冲液成份
Annexin V-PE (货号：10448-HNAE-P)	0.1 mL	0.5 mL	5 µL	含0.5% BSA、0.03% Proclin300的水溶液
7-AAD	0.1 mL	0.5 mL	5 µL	含0.5% BSA、0.03% Proclin300的水溶液
10× Binding Buffer	10 mL	50 mL	请参阅染色步骤	Aqueous buffered solution containing no preservative

应用验证

Annexin V-PE/7-AAD 凋亡检测试剂盒在喜树碱处理过的Jurkat细胞上做常规验证。Annexin V与磷脂酰丝氨酸（PS）的结合为钙离子依赖性的，在反应时需使用含钙离子的缓冲液。

Annexin V-PE/7-AAD 凋亡检测试剂盒未在贴壁细胞上做常规验证。在贴壁细胞的收集过程中，消化细胞容易造成细胞膜损伤，从而导致产生错误的实验结果。在您的研究中如果用到贴壁细胞，建议提前摸索细胞消化条件，并且尽量避免使用含EDTA的细胞消化液，以免产生错误的实验结果。



流式细胞术检测Annexin V-PE染色。Annexin V-PE及7-AAD在未处理的Jurkat细胞（左图）、4µM喜树碱（Sigma，货号C9911）处理5小时（中图）及18小时（右图）的Jurkat细胞上的染色结果。未处理的细胞主要为Annexin V-PE及7-AAD阴性。喜树碱处理5小时后，主要出现两群细胞：未凋亡的活细胞（Annexin V-PE、7-AAD双阴性），早期凋亡的细胞（Annexin V-PE 阳性、7-AAD阴性）。一小部分Annexin V-PE、7-AAD双阳性的细胞为凋亡晚期及已经死亡的细胞。经过18小时的处理后，凋亡晚期及已经死亡的细胞（Annexin V-PE、7-AAD双阳性）比例明显增加。

Annexin V-PE/7-AAD 凋亡检测试剂盒

货号: APK10448-P

Size: 20 Tests
100 Tests



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染色步骤

微量液体建议开盖前瞬时离心

1. 收集细胞, 用4℃预冷的PBS洗细胞2次, 离心去上清后用1×Binding Buffer重悬细胞至细胞密度为 $0.1 \times 10^7 \sim 1 \times 10^7$ 个/mL。
1×Binding Buffer: 1份10×Binding Buffer中加入9份蒸馏水, 混匀, 现用现配。
2. 将细胞悬液转移至流式管中, 每管100 μL.
3. 每管细胞中加入5 μL Annexin V-PE及5 μL 7-AAD, 轻轻混匀, 室温(25℃)避光孵育15min.
4. 加入400 μL 1X Binding Buffer终止反应, 1小时内上机检测。

注意事项

1. 为了得到准确的实验结果, 建议设置以下对照:
 - a) 阴性对照: 未染色的细胞。
 - b) 单染对照: 只染Annexin V-PE(无7-AAD)的细胞, 只染7-AAD(无Annexin V-PE)的细胞。
阴性对照和单染对照用于设置流式细胞仪电压及补偿。
 - c) 实验对照: 未处理的细胞, 用于确定本底水平的凋亡及死亡的细胞比例。
 - d) 选做对照: 为确定Annexin V-PE的结合特异性, 在加入Annexin V-PE之前, 可用纯化的Annexin V(10448-HNAE)封闭Annexin V-PE的结合位点。
2. 不要使用保质期外的试剂。
3. 避免混合使用不同批次的试剂。

背景

细胞凋亡是多细胞生物为维持内环境稳定, 由基因控制的细胞自主的有序的死亡过程。不同于细胞坏死, 细胞凋亡是主动的过程, 涉及一系列基因的激活、表达及调控。在凋亡过程中, 细胞会出现一系列形态学及生物化学性质的改变, 如质膜对称性改变、细胞皱缩、细胞核断裂、染色质凝聚、DNA断裂、mRNA降解等, 其中细胞质膜的改变是凋亡过程早期的重要事件之一。

Annexin V, 又称为Annexin A5 (ANXA5), 在细胞内含量丰富, 为高亲和力的 Ca^{2+} 依赖性磷脂(PLs)结合蛋白。在哺乳动物细胞中, 有多种类型的Annexin基因表达。在活细胞中, 磷脂酰丝氨酸(PS)主要定位于细胞质膜内侧, 在细胞凋亡的起始阶段, 磷脂酰丝氨酸从细胞内侧转移到细胞表面, 可与Annexin V高亲和力的结合。Annexin V与FITC或PE等荧光素偶联, 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。

7-氨基放线菌素D(7-AAD)是一种核酸染料, 不能通过正常细胞的细胞膜, 随着细胞凋亡、细胞死亡过程, 质膜对7-AAD的通透性逐渐增加, 7-AAD进入细胞与DNA结合。在流式细胞术中, 常用7-AAD鉴定死细胞, 这种作用与PI类似, 但它较PI有一个优点, 其发射波谱较PI窄, 对其他检测通道的干扰更小, 在多色荧光分析中是PI的最佳替代品。

在细胞凋亡的早期阶段, 磷脂酰丝氨酸暴露于细胞表面, 但细胞膜不具有通透性, 因此Annexin V可以与细胞结合, 但细胞拒染7-AAD, 因此早期凋亡的细胞为Annexin V阳性、7-AAD阴性。在凋亡晚期, 7-AAD可以通过细胞膜进入细胞与DNA结合, 同时Annexin V也可进入细胞, 与细胞内的磷脂酰丝氨酸结合, 此时细胞表型为Annexin V和7-AAD双阳性。但这种检测方法不能区分晚期凋亡与坏死的细胞, 因为无论是哪种途径死亡的细胞, 均表现为Annexin V和7-AAD双阳性。

	Annexin V	7-AAD
活细胞	—	—
早期凋亡细胞	+	—
晚期凋亡和死亡的细胞	+	+

+: 阳性
—: 阴性

参考文献

1. Cederholm A. et al., 2007, Ann N Y Acad Sci. 1108: 96-103.
2. Schlaepfer DD. et al., 1992, Biochemistry. 31:1886-91.
3. Vermes I. et al., 1995, J Immunol Methods. 184 (1): 39-51.