Annexin V-FITC /PI Assay Kit

***Storage*：**

Components	Storage
Binding Buffer	4°C
Annexin V-FITC	4°C
PI	4°C

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

IT IS RECOMMENDED THAT THE ENTIRE PROTOCOL BE REVIEWED BEFORE STARTING THE ASSAY.

Product Description

Apoptosis is a normal physiologic process which occurs during embryonic development as well as in maintenence of tissue homeostasis. The apoptotic program is characterized by certain morphologic features, including loss of plasma membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA. Loss of plasma membrane is one of the earliest features. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36 kDa Ca2+ dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS. Annexin V may be conjugated to fluorochromes including FITC. 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. Since externalization of PS occurs in the earlier stages of apoptosis, FITC Annexin V staining can identify apoptosis at an earlier stage than assays based on nuclear changes such as DNA fragmentation.

FITC Annexin V staining precedes the loss of membrane integrity which accompanies the latest stages of cell death resulting from either apoptotic or necrotic processes. Therefore, staining with FITC Annexin V is typically used in conjunction with a vital dye such as propidium iodide (PI) or 7-Amino-Actinomycin (7-AAD) to allow the investigator to identify early apoptotic cells (PI negative, FITC Annexin V positive). Viable cells with intact membranes exclude PI, whereas the membranes of dead and damaged cells are permeable to PI. For example, cells that are considered viable are both FITC Annexin V and PI negative while cells that are in early apoptosis are FITC Annexin V positive and PI negative, while cells that are in late apoptosis or already dead are both FITC Annexin V and PI positive. This assay does not distinguish between cells that have undergone apoptotic death versus those that have died as a result of a necrotic pathway because in either case, the dead cells will stain with both FITC Annexin V and PI. However, when apoptosis is measured over time, cells can be often tracked from

FITC Annexin V and PI negative (viable, or no measurable apoptosis), to FITC Annexin V positive and PI negative (early apoptosis, membrane integrity is present) and finally to FITC Annexin V and PI positive (end stage apoptosis and death). The movement of cells through these three stages suggests apoptosis. In contrast, a single observation indicating that cells are both FITC Annexin V and PI positive, in of itself, reveals less information about the process by which the cells underwent their demise.
FITC Annexin V is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.

Protocol:

FITC Annexin V is used to quantitatively determine the percentage of cells within a population that are actively undergoing apoptosis. It relies on the property of cells to lose membrane asymmetry in the early phases of apoptosis. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner leaflet of the plasma membrane to the outer leaflet, thereby exposing PS to the external environment. Annexin V is a calcium-dependent phospholipid-binding protein that has a high affinity for PS, and is useful for identifying apoptotic cells with exposed PS. Propidium Iodide (PI) is a standard flow cytometric viability probe and is used to distinguish viable from nonviable cells. Viable cells with intact membranes exclude PI, whereas the membranes of dead and damaged cells are permeable to PI. Cells that stain positive for FITC Annexin V and negative for PI are undergoing apoptosis. Cells that stain positive for both FITC Annexin V and PI are either in the end stage of apoptosis, are undergoing necrosis, or are already dead. Cells that stain negative for both FITC Annexin V and PI arealive and not undergoing measurable apoptosis.

Reagents

1. FITC Annexin V: Included. Use 5 μl per test.
2. Propidium Iodide (PI): Ready-to-use nucleic acid dye. Use up to 5-10 μl per test solution.
3. Binding Buffer: Ready-to-use

Staining

1. **Suspension cell:** Centrifuge cells at 300-500g, 2-8 ° C, 5min, discard the culture medium
   Adherent cell: Digested cells with trypsin without EDTA and centrifuged (300-500g, 2-8 ° C, 5min) to collect cells
2. Wash cells twice with cold PBS and then resuspend cells in 400 μl Binding Buffer at a concentration of 1 × 10^6 cells/ml.
3. Add 5 μl of FITC Annexin V and protected from light in 4° C, 15min.
4. Add 10 μl PI. and protected from light in 4° C, 5min.
5. Gently vortex the cells and incubate for 15 min at RT (25 ° C) in the dark.
6. Analyze by flow cytometry within 1 hr.

SUGGESTED CONTROLS FOR SETTING UP FLOW CYTOMETRY

The following controls are used to set up compensation and quadrants:

1. Unstained cells.
2. Cells stained with FITC Annexin V (no PI).
3. Cells stained with PI (no FITC Annexin V).

Other Staining Controls:

A cell line that can be easily induced to undergo apoptosis should be used to obtain positive control staining with

FITC Annexin V and/or FITC Annexin V and PI. It is important to note that the basal level of apoptosis and necrosis varies considerably within a population. Thus, even in the absence of induced apoptosis, most cell populations will contain a minor percentage of cells that are positive for apoptosis (FITC Annexin V positive, PI negative or FITC Annexin V positive, PI positive). The untreated population is used to define the basal level of apoptotic and dead cells. The percentage of cells that have been induced to undergo apoptosis is then determined by subtracting the percentage of apoptotic cells in the untreated population from percentage of apoptotic cells in the treated population. Since cell death is the eventual outcome of cells undergoing apoptosis, cells in the late stages of apoptosis will have a damaged membrane and stain positive for PI as well as for FITC Annexin V. Thus the assay does not distinguish between cells that have already undergone an apoptotic

Annexin V-FITC 细胞凋亡检测试剂盒

产品组成：

Annexin V-FITC 染色液	500 μL	4° C 保存
PI 染色液	1000 μL	4° C 保存
结合液	80mL	4° C 保存

储存条件：

2-8℃避光保存。

长期不用可以分装后-20℃避光保存延长有效期，避免反复冻融。

有效期：

一年。

产品简介：

产品背景：

细胞凋亡是细胞的基本特征之一，它在机体的胚胎发育、组织修复、内环境的稳定和一些疾病发生过程等方面起着十分重要的作用。在正常细胞中，磷脂酰丝氨酸（PS）只分布在细胞膜脂质双层的内侧，而在细胞凋亡早期，细胞膜中的磷脂酰丝氨酸（PS）由脂膜内侧翻向外侧。在体内，巨噬细胞可以识别翻转到细胞膜表面的 PS 从而将这些程序性死亡的细胞清除，因此凋亡过程中并不伴随局部的炎症反应，而在细胞坏死的过程中则常常伴随着炎症反应。

Annexin V 是一种分子量为 35-36kD 的 Ca^{2+}依赖性磷脂结合蛋白，能与细胞凋亡过程中翻转到膜外的 PS 高亲和力特异性结合。PS 外翻发生在细胞核破裂，DNA 片段化以及凋亡相关蛋白出现之前，这使得 Annexin V 与 PS 的结合成为凋亡早期的一种重要检测标志事件。

检测方法：

流式细胞仪或荧光显微镜

样本类型：

● 悬浮细胞　　● 贴壁细胞

使用方法：

- 由于细胞凋亡是一个快速和动态的过程，因此最好在染色后立即进行分析。
- 使用 Annexin V-FITC 试剂盒检测凋亡需要针对活细胞。不要固定细胞，固定操作会对结果产生干扰。
- 流式细胞仪检测时，细胞数量应不低于 1×10^5。
- 如果细胞收集过程中使用了胰酶，需注意用 PBS 洗净去除残留的胰酶。残留的胰酶会消化并降解 Annexin V-FITC，最终导致染色失败。

样品染色：

1、 离心收集悬浮细胞。离心机 300-500g，2-8℃，离心时间 5min，弃培养基。（贴壁细胞用不含 EDTA 的胰酶消化后离心，收集细胞。胰酶消化时间不宜过长，以防引起假阳性）。
2、 用冷 PBS 洗涤细胞两次。（300g，2-8℃，离心时间 5min 收集细胞）。
3、 用 400ul 1X Annexin V 结合液悬浮细胞，浓度大约为 $1 X 10^6$ cells/ml。
4、 在细胞悬浮液中加入 5ul Annexin V-FITC 染色液，轻轻混匀后于 2-8℃ 避光条件下孵育 15 分钟。
5、 加入 5-10ul PI 染色液后轻轻混匀于 2-8℃ 避光条件下孵育 5 分钟。
6、 立即用流式细胞仪或荧光显微镜检测。

流式细胞仪分析：

经处理过的细胞此时可以在流式细胞仪上分析。激发波长为 488nm。

按照常规的流式凋亡检测的操作即可。

Annexin V-FITC 的绿色荧光发射波长 530nm，信号可以通过 FL1（FITC 接收器）通道检测；PI 红色荧光在 620nm，通过 FL2（Propidium iodide 接收器）通道或 FL3 通道检测。

上机检测前，需用待测细胞制备三个质控样本来设定流式细胞仪的荧光补偿和设置十字门的范围：

① 没有染色的细胞;
② 仅用 Annexin V-FITC 染色的细胞;
③ 仅用 PI 染色的细胞.

荧光显微镜观察：

1. 滴加 30-50ul 用 Annexin V-FITC/PI 双染的细胞悬液于载玻片上，并用盖玻片盖上细胞。

   注：对于贴壁细胞，可以象悬浮细胞那样染色后， 滴一滴细胞悬液于载玻片上，用盖玻片盖上细胞，荧光显微镜下观察。也可直接用盖玻片培养细胞并诱导细胞凋亡。根据盖玻片大小，置于 24 孔或 12 孔细胞培养板内培养，然后诱导细胞凋亡。细胞染色在细胞培养板内进行。先用 PBS 冲洗两次，加入 400μl Annexin V 结合液于孔中。再加入 5μl Annexin V-FITC 染色液与 10μl Propidium Iodide 染色液，混匀。避光室温反应 10 分钟。

2. 在荧光显微镜下用双色滤光片观察。使用荧光显微镜上的 FITC 滤镜（蓝光），Annexin V-FITC 染色阳性的细胞将在细胞膜表面呈现明亮的苹果绿色。使用 Rhodamine 滤镜（绿光），Propidium Iodide 染色阳性的细胞则会在整个胞质内呈现不同强度的黄-红色。早期凋亡细胞不会被 Propidium Iodide 染色或显示背景荧光，而坏死或晚期凋亡细胞则会显示出黄-红色的胞质，红色的胞核和环绕细胞的绿色胞膜。在晚期凋亡细胞中还可以观察到胞膜皱缩和起泡。