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Annexin V-FITC细胞凋亡检测试剂盒

产品编号	产品名称	包装
C1062S	Annexin V-FITC细胞凋亡检测试剂盒	20次
C1062M	Annexin V-FITC细胞凋亡检测试剂盒	50次
C1062L	Annexin V-FITC细胞凋亡检测试剂盒	100次

产品简介：

- 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是一类广泛分布于真核细胞细胞浆内钙离子依赖的磷脂结合蛋白，参与细胞内的信号转导。但仅Annexin V被报道可以调控一些PKC的活性。
- 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。
- 本试剂盒的流式检测效果参考图1和图2，荧光显微镜检测效果参考图3。

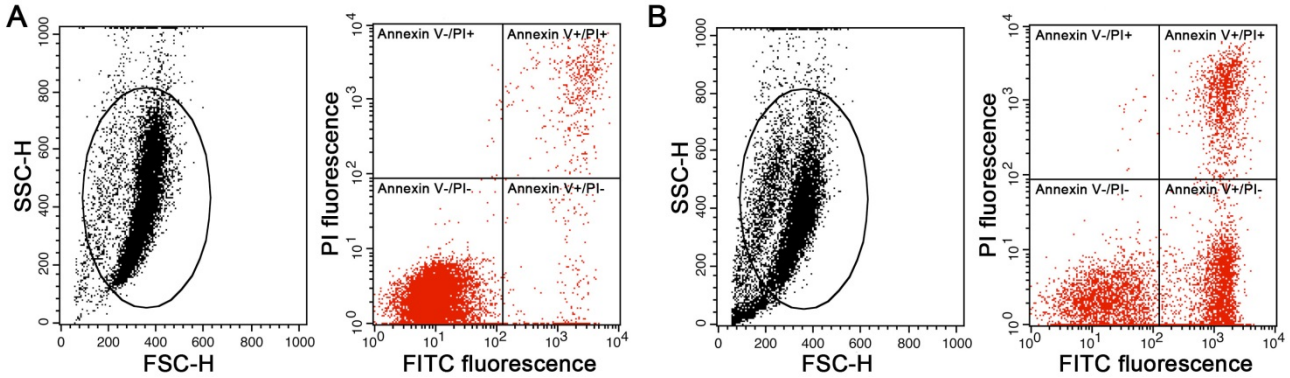

图1. 细胞用本试剂盒染色后流式细胞仪检测细胞凋亡的效果图。Jurkat细胞未经处理(A)或用10μM喜树碱(Camptothecin)作用4小时(B)后，用本试剂盒染色，然后用流式细胞仪进行细胞的散射和荧光检测。从图中可以看出，经过凋亡诱导剂喜树碱处理后的细胞，其Annexin V-FITC染色阳性并且PI染色阴性的细胞，即凋亡细胞，明显增加(B图的右下象限)，Annexin V-FITC和PI染色双阳性的细胞，即坏死细胞，也有所增加(B图的右上象限)。图中Annexin V-FITC染色阴性PI染色阳性(Annexin V-/PI+)所在象限(A和B图的左上象限)出现的细胞点是许可范围内的检测误差。实测数据可能会因细胞类型、细胞凋亡情况、检测仪器等的不同而存在差异，图中数据仅供参考。

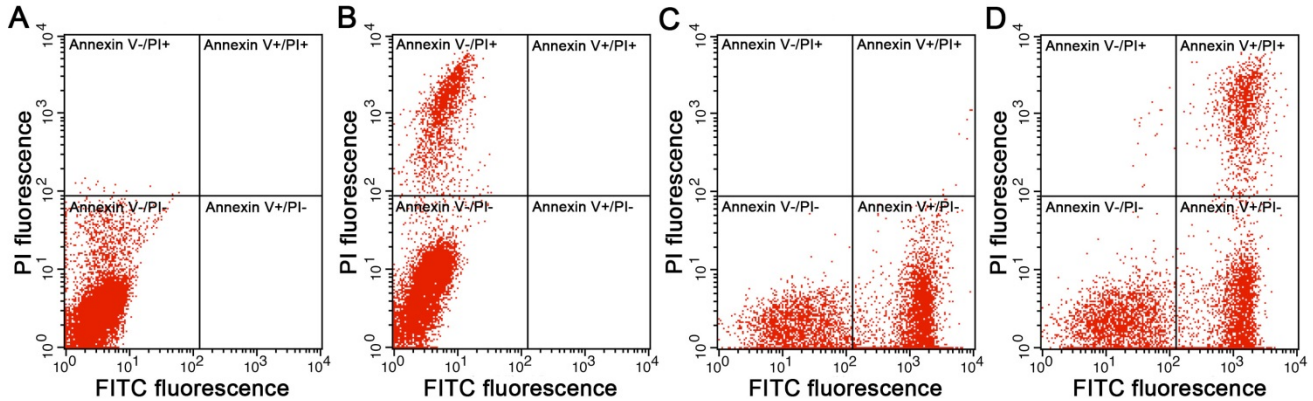

图2. 细胞用本试剂盒染色后流式细胞仪检测效果验证图。Jurkat细胞用10μM喜树碱(Camptothecin)作用4小时后，未经染色(A)、仅用本试剂盒中的PI进行染色(B)、仅用本试剂盒中的Annexin V-FITC进行染色(C)、用本试剂盒中的Annexin V-FITC和PI进行双染(D)。从图中可以看出，未经染色的是双阴性细胞(A)，仅PI染色后出现了预期的PI染色阳性的细胞，仅Annexin V-FITC染色出现了预期的Annexin V-FITC染色阳性的细胞，Annexin V-FITC和PI双染出现了预期的仅Annexin V-FITC染色阳性的凋亡细胞和双阳性的坏死细胞。出现的非预期的细胞点完全在许可的误差范围内。实测数据可能会因细胞类型、细胞凋亡情况、检测仪器等的不同而存在差异，图中数据仅供参考。

- 本试剂盒小包装C1062S可以检测20个样品，中包装C1062M可以检测50个样品，大包装C1062L可以检测100个样品。

包装清单：

产品编号	产品名称	包装
C1062S-1	Annexin V-FITC	100μl
C1062S-2	Annexin V-FITC结合液	12ml
C1062S-3	碘化丙啶染色液	220μl
—	说明书	1份

产品编号	产品名称	包装
C1062M-1	Annexin V-FITC	250μl
C1062M-2	Annexin V-FITC结合液	30ml
C1062M-3	碘化丙啶染色液	550μl
—	说明书	1份

产品编号	产品名称	包装
C1062L-1	Annexin V-FITC	500μl
C1062L-2	Annexin V-FITC结合液	60ml
C1062L-3	碘化丙啶染色液	1.1ml
—	说明书	1份

保存条件：

4ºC保存，半年有效。-20ºC保存，一年有效。Annexin V-FITC和碘化丙啶染色液需要避光保存。为了长期保存，可以把碘化丙啶染色液适当分装后-20ºC保存。

注意事项：

- 尽管经测试Annexin V-FITC反复冻融5次对于其检测效果无显著影响，但为取得良好的使用效果，3-6个月内推荐4ºC保存，并适当注意避免反复冻融。
- 如果有细菌或真菌污染，会严重影响检测效果。
- 染色后宜尽快检测，时间过长可能会导致凋亡或坏死细胞的数量增加。
- 如果细胞收集过程中使用了胰酶，需注意设法去除残留的胰酶。残留的胰酶会消化并降解Annexin V-FITC，导致染色失败。
- 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。
- 用于流式细胞仪检测时，如果发现Annexin V-FITC单独染色时出现了过多的PI假阳性细胞，并且通过调整相关设置和参数也无法改善，可以用PBS将Annexin V-FITC稀释3-10倍后再进行检测，这样通常可以有效减少假阳性的坏死细胞。
- 需自备PBS。
- 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. 对于悬浮细胞：

a. 在进行完细胞凋亡刺激后，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：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195μl Annexin V-FITC结合液轻轻重悬细胞。

c. 加入5μl Annexin V-FITC，轻轻混匀。

d. 加入10μl碘化丙啶染色液，轻轻混匀。

e. 室温(20-25ºC)避光孵育10-20分钟，随后置于冰浴中。可以使用铝箔进行避光。孵育过程中可以重悬细胞2-3次以改善染色效果。

f. 如果用于流式细胞仪检测，可立即上机检测，Annexin V-FITC为绿色荧光，碘化丙啶(PI)为红色荧光，流式检测细胞凋亡的效果及其验证请参考图1和图2。初次进行流式细胞仪检测时，建议选择一组适当的细胞参考图2设置未染色、PI单染和Annexin V-FITC单染这3个对照。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。注意：细胞在染色后须尽快完成检测，通常宜在1小时之内完成检测。用于流式细胞仪检测时，如果发现Annexin V-FITC单独染色时出现了过多的PI假阳性细胞，并且通过调整相关设置和参数也无法改善，可以用PBS将Annexin V-FITC稀释3-10倍后再进行检测。

2. 对于贴壁细胞的消化后检测：

a. 把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(可含有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。注意：对于贴壁细胞，胰酶消化步骤很关键。胰酶消化时间如果过短，细胞需要用力吹打才能脱落，容易造成细胞膜的损伤，从而导致细胞坏死的假阳性；消化时间如果过长，同样易造成细胞膜损伤而出现细胞坏死的假阳性，甚至会影响细胞膜上磷脂酰丝氨酸与Annexin V-FITC的结合从而干扰对于细胞凋亡的检测。同时，胰酶细胞消化液中应尽量不含EDTA，因为EDTA可能会影响Annexin V与磷脂酰丝氨酸的结合。

b. 加入步骤2a中收集的细胞培养液，把细胞轻轻吹打下来，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中的细胞培养液非常重要，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抑制或中和残留的胰酶。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195μl Annexin V-FITC结合液轻轻重悬细胞。

d. 加入5μl Annexin V-FITC，轻轻混匀。

e. 加入10μl碘化丙啶染色液，轻轻混匀。

f. 室温(20-25ºC)避光孵育10-20分钟，随后置于冰浴中。可以使用铝箔进行避光。孵育过程中可以重悬细胞2-3次以改善染色效果。

g. 如果用于流式细胞仪检测，可立即上机检测，Annexin V-FITC为绿色荧光，碘化丙啶(PI)为红色荧光，流式检测的效果及其验证请参考图1和图2。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。注意：细胞在染色后须尽快完成检测，通常宜在1小时之内完成检测。用于流式细胞仪检测时，如果发现Annexin V-FITC单独染色时出现了过多的PI假阳性细胞，并且通过调整相关设置和参数也无法改善，可以用PBS将Annexin V-FITC稀释3-10倍后再进行检测。

3. 对于贴壁细胞的原位荧光检测：

注：本方法的优点是可以原位观察细胞凋亡，缺点是部分凋亡由于不贴壁而检测不到。

a. **(选做)**如果条件许可，把细胞培养于24孔板、48孔板或96孔板内。在凋亡诱导结束后，用可以对多孔板进行离心的离心机1000g离心5分钟。

b. 吸除细胞培养液，加入PBS洗涤一次。如果条件许可，在吸除PBS前1000g离心5分钟。

c. 加入195μl Annexin V-FITC结合液。

d. 加入5μl Annexin V-FITC，轻轻混匀。

e. 加入10μl碘化丙啶染色液，轻轻混匀。

f. 室温(20-25ºC)避光孵育10-20分钟，随后置于冰浴中。可以使用铝箔进行避光。

g. 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。注意：细胞在染色后须尽快完成检测，通常宜在1小时之内完成检测。

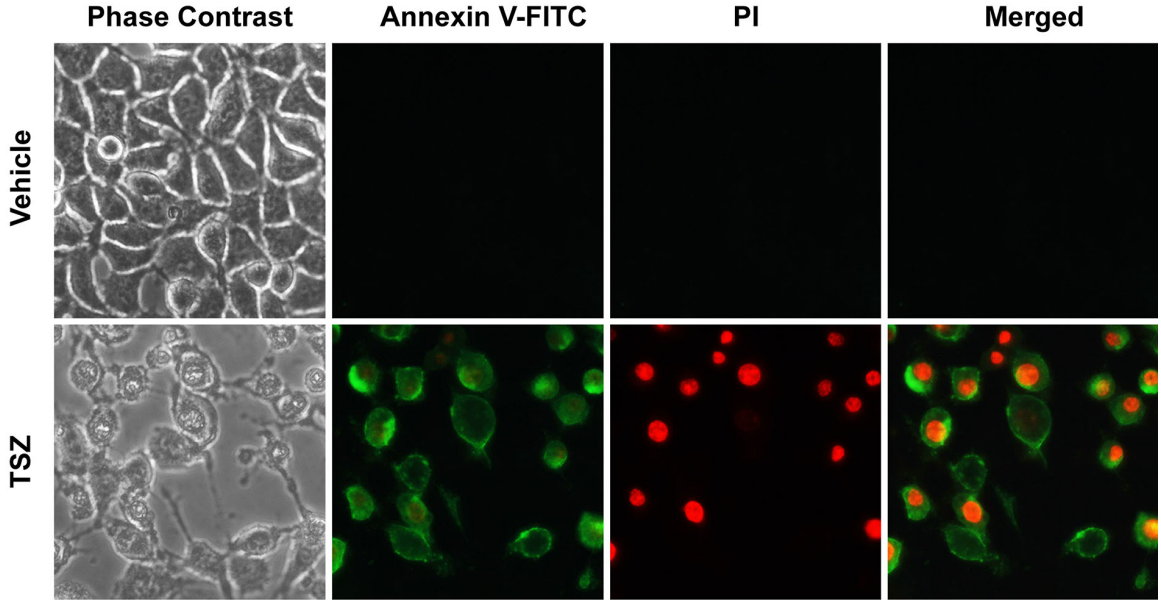

图3. Annexin V-FITC和碘化丙啶(PI)染色效果图。图中绿色荧光为Annexin V-FITC染色阳性细胞，红色荧光为碘化丙啶染色阳性细胞。仅被绿色荧光染色的为凋亡细胞，被绿色和红色荧光双染的是坏死细胞，未被荧光染色的为正常细胞。Vehicle组为阴性对照，Annexin V-FITC染色和碘化丙啶染色都非常弱，L-929细胞处于正常状态；TSZ组为阳性对照，L-929细胞用TSZ处理3小时。TSZ为TNFα、SM-164和Z-VAD-FMK组成的细胞坏死诱导试剂(C1058)。

相关产品：

产品编号	产品名称	包装
C1052	细胞周期与细胞凋亡检测试剂盒	50次
C1056	细胞凋亡与坏死检测试剂盒	100次
C1062S	Annexin V-FITC细胞凋亡检测试剂盒	20次
C1062M	Annexin V-FITC细胞凋亡检测试剂盒	50次
C1062L	Annexin V-FITC细胞凋亡检测试剂盒	100次
C1065S	Annexin V-PE细胞凋亡检测试剂盒	20次
C1065M	Annexin V-PE细胞凋亡检测试剂盒	50次
C1065L	Annexin V-PE细胞凋亡检测试剂盒	100次
C1067S	Annexin V-EGFP细胞凋亡检测试剂盒	20次
C1067M	Annexin V-EGFP细胞凋亡检测试剂盒	50次
C1082	TUNEL检测阳性对照制备试剂盒	10次
C1086	一步法TUNEL细胞凋亡检测试剂盒(绿色荧光)	20次
C1088	一步法TUNEL细胞凋亡检测试剂盒(绿色荧光)	50次
C1089	一步法TUNEL细胞凋亡检测试剂盒(红色荧光)	20次
C1090	一步法TUNEL细胞凋亡检测试剂盒(红色荧光)	50次
C1091	TUNEL细胞凋亡检测试剂盒(显色法)	20次
C1098	TUNEL细胞凋亡检测试剂盒(显色法)	50次

使用本产品的文献：

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