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细胞周期与细胞凋亡检测试剂盒

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

产品简介：

- 碧云天生产的细胞周期与细胞凋亡检测试剂盒(Cell Cycle and Apoptosis Analysis Kit)是一种采用经典的碘化丙啶染色(Propidium staining，即PI staining)方法进行细胞周期与细胞凋亡分析的检测试剂盒。
- 碘化丙啶(Propidium，简称PI)是一种双链DNA的荧光染料。碘化丙啶和双链DNA结合后可以产生荧光，并且荧光强度和双链DNA的含量成正比。细胞内的DNA被碘化丙啶染色后，可以用流式细胞仪对细胞进行DNA含量测定，然后根据DNA含量的分布情况，可以进行细胞周期和细胞凋亡分析。
- 碘化丙啶染色后，假设G0/G1期细胞的荧光强度为1，那么含有双份基因组DNA的G2/M期细胞的荧光强度的理论值为2，正在进行DNA复制的S期细胞的荧光强度为1-2之间。凋亡细胞由于细胞核发生浓缩以及发生DNA片段化(DNA fragmentation)导致部分基因组DNA片段在染色过程中丢失，因此凋亡细胞碘化丙啶染色后呈现明显的弱染，即荧光强度小于1，在流式检测的荧光图上出现所谓的sub-G1峰，即凋亡细胞峰。
- 细胞发生凋亡时，由于胞浆和染色质浓缩、核碎裂，产生凋亡小体，使细胞的光散射性质发生变化。在细胞凋亡的早期，细胞对前向角光散射的能力显著降低，对侧向光散射的能力增加或没有变化。在细胞凋亡的晚期，前向和侧向光散射的信号均降低。因此可通过流式细胞仪测定细胞光散射的变化观察细胞凋亡情况。使用本试剂盒检测经凋亡诱导的HeLa细胞的效果请参考图1。

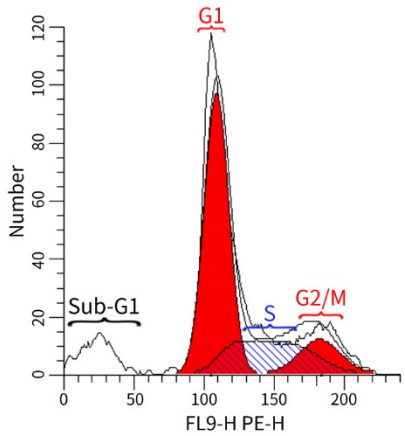

图1. 碧云天细胞周期与细胞凋亡检测试剂盒(C1052)检测经凋亡诱导的HeLa细胞的效果图。HeLa细胞经适当凋亡诱导后，用70%乙醇固定，并经本试剂盒染色后进行流式检测以分析细胞周期和细胞凋亡。图中可见凋亡细胞的Sub-G1峰。实际检测效果会因实验条件、检测仪器等的不同而存在差异，图中效果仅供参考。

- 本试剂盒通常应用于培养的贴壁或悬浮细胞的细胞周期与细胞凋亡检测。如果用于组织的细胞周期与细胞凋亡检测，则必须把组织消化成单细胞状态，才可以进行检测。
- 本试剂盒足够检测50个样品，每个样品的细胞数量可以为10-100万。

包装清单：

产品编号	产品名称	包装
C1052-1	染色缓冲液	25ml
C1052-2	碘化丙啶染色液(20X)	1.25ml
C1052-3	RNase A (50X)	0.5ml
—	说明书	1份

保存条件：

-20°C保存，一年有效。C1052-2碘化丙啶染色液(20X)需避光保存。本试剂盒可4°C保存，一个月内有效。

注意事项：

- 本试剂盒需使用流式细胞仪进行检测。

- 需自备PBS和70%乙醇，PBS (C0221A)可以向碧云天订购。
- 荧光染料均存在淬灭问题，保存和使用过程中请尽量注意避光，以减缓荧光淬灭。
- 碘化丙啶对人体有刺激性，操作时请小心，并注意适当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. 细胞样品的准备：
   a. 对于贴壁细胞：小心收集细胞培养液到一离心管内备用。用胰酶消化细胞，至细胞可以被轻轻用移液管或枪头吹打下来时，加入前面收集的细胞培养液，吹打下所有的贴壁细胞，并轻轻吹散细胞。再次收集到离心管内。1000g左右离心3-5分钟，沉淀细胞。对于特定的细胞，如果细胞沉淀不充分，可以适当延长离心时间或稍稍加大离心力。小心吸除上清，可以残留约50微升左右的培养液，以避免吸走细胞。加入约1毫升冰浴预冷的PBS，重悬细胞，并转移到1.5毫升离心管内。再次离心沉淀细胞，小心吸除上清，可以残留约50微升左右的PBS，以避免吸走细胞。轻轻弹击离心管底以适当分散细胞，避免细胞成团。
   b. 对于悬浮细胞：1000g左右离心3-5分钟，沉淀细胞。对于特定的细胞，如果细胞沉淀不充分，可以适当延长离心时间或稍稍加大离心力。小心吸除上清，可以残留约50微升左右的培养液，以避免吸走细胞。加入约1毫升冰浴预冷的PBS，重悬细胞，并转移到1.5毫升离心管内。再次离心沉淀细胞，小心吸除上清，可以残留约50微升左右的PBS，以避免吸走细胞。轻轻弹击离心管底以适当分散细胞，避免细胞成团。
2. 细胞固定：加入1毫升冰浴预冷70%乙醇中，轻轻吹打混匀，4°C固定30分钟或更长时间。通常固定2小时或以上更能保证染色效果，固定12-24小时可能效果更佳。1000g左右离心3-5分钟，沉淀细胞。对于特定的细胞，如果细胞沉淀不充分，可以适当延长离心时间或稍稍加大离心力。小心吸除上清，可以残留约50微升左右的70%乙醇，以避免吸走细胞。加入约1毫升冰浴预冷的PBS，重悬细胞。再次离心沉淀细胞，小心吸除上清，可以残留约50微升左右的PBS，以避免吸走细胞。轻轻弹击离心管底以适当分散细胞，避免细胞成团。
3. 碘化丙啶染色液的配制：参考下表，根据待检测样品的数量配制适量的碘化丙啶染色液：

	1个样品	6个样品	12个样品
染色缓冲液	0.5ml	3ml	6ml
碘化丙啶染色液(20X)	25μl	150μl	300μl
RNase A (50X)	10μl	60μl	120μl
Final volume	0.535ml	3.21ml	6.42ml

   注：配制好的碘化丙啶染色液短时间内可以4°C保存，宜当日使用。
4. 染色：每管细胞样品中加入0.5毫升碘化丙啶染色液，缓慢并充分重悬细胞沉淀， 37°C避光温浴30分钟。随后可以4°C或冰浴避光存放。染色完成后宜在24小时内完成流式检测，最好能在当日完成流式检测。
5. 流式检测和分析：用流式细胞仪在激发波长488nm波长处检测红色荧光，同时检测光散射情况。采用适当分析软件进行细胞DNA含量分析和光散射分析。

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