

## 一步法TUNEL细胞凋亡检测试剂盒

产品编号	产品名称	包装
C1086	一步法TUNEL细胞凋亡检测试剂盒(绿色荧光)	20次

### 产品简介:

- 碧云天生产的一步法TUNEL细胞凋亡检测试剂盒(One Step TUNEL Apoptosis Assay Kit)为您提供了一种高灵敏度又快速简便的细胞凋亡检测方法。对于经过固定和洗涤的细胞或组织，只要经过一步染色反应，洗涤后就可以通过荧光显微镜或流式细胞仪检测到呈现绿色荧光的凋亡细胞。
- 细胞在发生凋亡时，会激活一些DNA内切酶，这些内切酶会切断核小体间的基因组DNA。细胞凋亡时抽提DNA进行电泳检测，可以发现180-200bp的DNA ladder。基因组DNA断裂时，暴露的3'-OH可以在末端脱氧核苷酸转移酶(Terminal Deoxynucleotidyl Transferase, TdT)的催化下加上绿色荧光探针荧光素(FITC)标记的dUTP(fluorescein-dUTP)，从而可以通过荧光显微镜或流式细胞仪进行检测，这就是TUNEL(TdT-mediated dUTP Nick-End Labeling)法检测细胞凋亡的原理。  
注：FITC是fluorescein isothiocyanate的缩写，实际上大多数情况下所谓的FITC即为fluorescein。
- 本试剂盒有如下优点。(1) 高灵敏度：背景染色极低，阳性染色明亮，可以在单细胞水平检测到细胞凋亡，同时由于凋亡早期就有DNA断裂，可以检测到早期的细胞凋亡。(2) 特异性：TUNEL检测时通常更容易标记凋亡细胞，而不容易标记坏死细胞。(3) 快速：仅需约1-2个小时即可完成。(4) 方便：只需一步染色反应，洗涤后即可观察，不必使用二抗等进行多步操作。(5) 应用范围广：可以用于检测冷冻或石蜡切片中的细胞凋亡情况，也可以检测培养的贴壁细胞或悬浮细胞的凋亡情况。(6) 实测效果好：参考图1，本试剂盒的实际检测效果和国外同类产品相当。

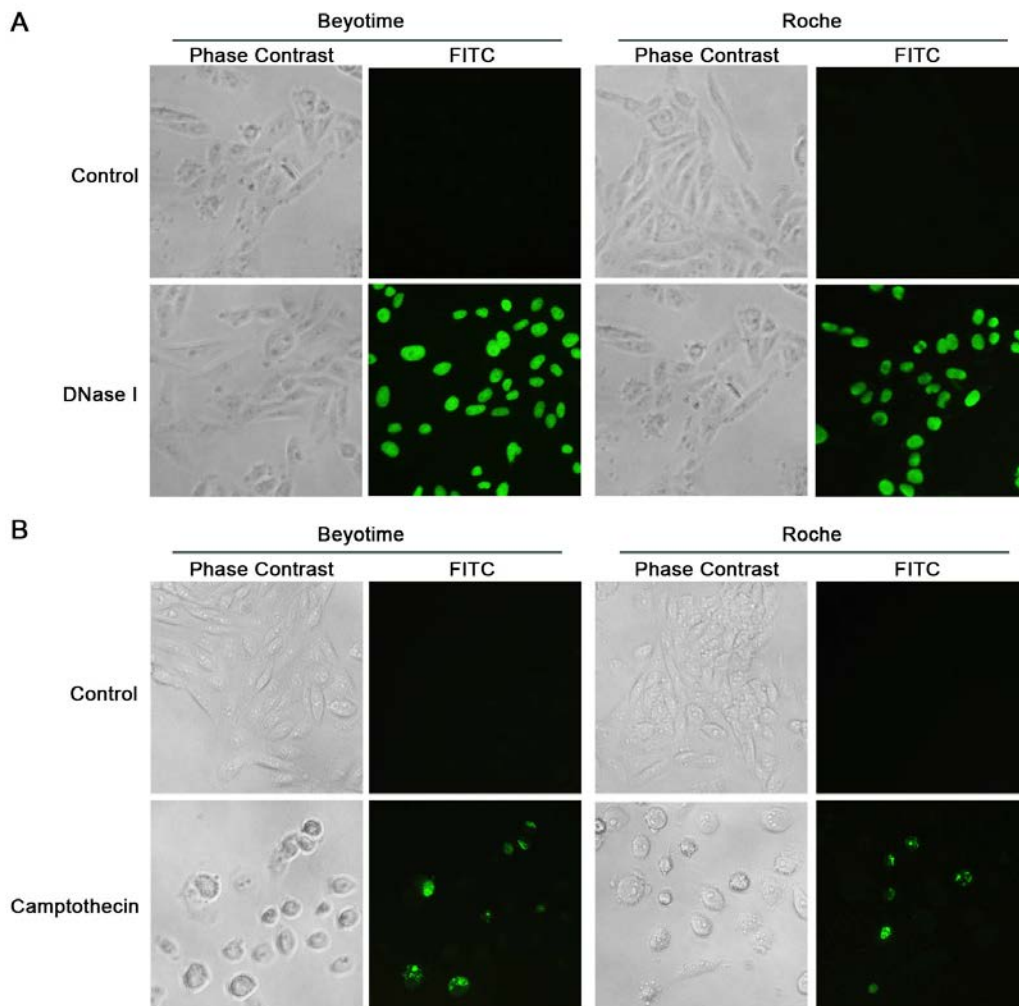


图1. 本产品的检测效果以及和Roche公司同类产品检测效果的比较。A. 碧云天的本试剂盒和Roche公司同类试剂盒分别检测正

常HeLa细胞和经DNase I处理的HeLa细胞的效果图，图中的绿色荧光为TUNEL染色阳性细胞。B. 碧云天的本试剂盒和Roche公司同类试剂盒分别检测正常HeLa细胞和经10 $\mu$ M喜树碱(Camptothecin)处理24小时的HeLa细胞的效果图。无论是经DNase I处理的阳性细胞样品，还是使用10 $\mu$ M喜树碱处理的阳性细胞样品，碧云天的本试剂盒和Roche公司的同类试剂盒的效果相当。本图中的染色实验均在12孔板中进行。本图仅作参考，不同的样品不同的检测条件，实际获得的结果可能有较明显的差别。

- TUNEL法特异性检测细胞凋亡时产生的DNA断裂，但不会检测出射线等诱导的DNA断裂(和细胞凋亡时的断裂方式不同)。这样一方面可以把凋亡和坏死区分开，另一方面也不会把射线等诱导发生DNA断裂的非凋亡细胞判断为凋亡细胞。
- 极少数细胞凋亡时没有DNA断裂，此时不适用TUNEL法检测。在个别类型的坏死细胞中也发现TUNEL检测呈阳性。在需要严格判断细胞凋亡的情况下，最好同时检测多个凋亡指标。
- 本试剂盒足够检测20个样品。

#### 包装清单：

产品编号	产品名称	包装
C1086-1	TdT酶	100 $\mu$ l
C1086-2	荧光标记液	900 $\mu$ l
C1086-3	TdT酶稀释液(选用)	500 $\mu$ l
—	说明书	1份

#### 保存条件：

-20 $^{\circ}$ C保存，荧光标记液需避光保存。

#### 注意事项：

- 需自备用于洗涤细胞的PBS或HBSS，用于封片的抗荧光淬灭封片液(P0126)，用于固定的4%多聚甲醛或向碧云天订购免疫染色固定液(P0098)，同时需自备含0.3% Triton X-100的PBS或向碧云天订购免疫染色强力通透液(P0097)。
- 如果用于石蜡切片的检测，需自备蛋白酶K，二甲苯。蛋白酶K(ST533)可以向碧云天订购。
- 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. 对于贴壁细胞或细胞涂片：

- a. PBS或HBSS洗涤一次。
- b. 如果细胞贴得不牢，可以干燥样品使细胞贴得更牢。
- c. 用碧云天生产的免疫染色固定液(P0098)或4%多聚甲醛固定细胞30分钟。
- d. 用PBS或HBSS洗涤一次。
- e. 加入碧云天生产的免疫染色强力通透液(P0097)或含0.3% Triton X-100的PBS，室温孵育5分钟。
- f. 转步骤5。

##### 2. 对于悬浮细胞或细胞悬液：

- a. 收集细胞(不超过200万细胞)，PBS或HBSS洗涤一次。
- b. 用碧云天生产的免疫染色固定液(P0098)或4%多聚甲醛固定细胞30分钟。为防止细胞聚集成团，宜在侧摆摇床或水平摇床上缓慢摇动的同时进行固定。
- c. 用PBS或HBSS洗涤一次。
- d. 用碧云天生产的免疫染色强力通透液(P0097)或含0.3% Triton X-100的PBS重悬细胞，室温孵育5分钟。
- e. 转步骤5。

##### 3. 对于石蜡切片：

- a. 二甲苯中脱蜡5-10分钟。换用新鲜的二甲苯，再脱蜡5-10分钟。无水乙醇5分钟。90%乙醇2分钟。70%乙醇2分钟，蒸馏水2分钟。
- b. 滴加20 $\mu$ g/ml不含DNase的蛋白酶K(推荐使用碧云天的ST532/ST533 蛋白酶K(20mg/ml)，用P0106 免疫染色洗涤液或10mM Tris-HCl pH7.4-7.8稀释1000倍即为20 $\mu$ g/ml不含DNase的蛋白酶K)，20-37 $^{\circ}$ C作用15-30分钟(不同组织的最佳作用温度和时间需自行摸索)。
- c. PBS或HBSS洗涤3次。注意：这一步必须把蛋白酶K洗涤干净，否则会严重干扰后续的标记反应。
- d. 转步骤5。

##### 4. 对于冰冻切片：

- a. 用碧云天生产的免疫染色固定液(P0098)或4%多聚甲醛固定细胞30-60分钟。
- b. PBS或HBSS洗涤2次，每次10分钟。
- c. 加入碧云天生产的免疫染色强力通透液(P0097)或含0.5% Triton X-100的PBS，室温孵育5分钟。
- d. 转步骤5。

##### 5. 配制TUNEL检测液：

参考下表配制适量的TUNEL检测液，需充分混匀。注意：配制好的TUNEL检测液必须一次使用完毕，不能冻存。

	1个样品	5个样品	10个样品
TdT酶	5 $\mu$ l	25 $\mu$ l	50 $\mu$ l
荧光标记液	45 $\mu$ l	225 $\mu$ l	450 $\mu$ l
TUNEL检测液	50 $\mu$ l	250 $\mu$ l	500 $\mu$ l

#### 6. 对于贴壁细胞、细胞涂片或组织切片：

- 用PBS或HBSS洗涤2次。
- 在样品上加50 $\mu$ l TUNEL检测液，37 $^{\circ}$ C避光孵育60分钟。注意：50 $\mu$ l TUNEL检测液适合涂片、切片或96孔板、48孔板、24孔板或12孔板的一个孔，如果是6孔板中的一个孔TUNEL检测液宜使用100 $\mu$ l。如果待检测的样品为涂片、切片或在24孔板、12孔板或6孔板中，可以使用防蒸发膜，或自行尝试使用自封袋或者其它适当材料自行裁剪成比孔略小的圆形塑料片，滴加TUNEL检测液后覆盖在样品上，可以防止TUNEL检测液蒸发，并且使TUNEL检测液均匀覆盖样品。自行裁剪圆片时需要连着圆片突出一个角或连着一边，并将圆形之外的突出部分折叠，方便染色结束后利用突出部分顺利取出圆片。也可以用PAP Pen圈出一个区域进行染色。孵育时需注意在多余的孔和多孔板的空隙中加入适量水以保持湿润，从而尽量减少TUNEL检测液的蒸发。
- PBS或HBSS洗涤3次。
- 用抗荧光淬灭封片液封片后荧光显微镜下观察。可以使用的激发波长范围为450-500nm，发射波长范围为515-565nm(绿色荧光)。染色效果可参见图1。

#### 7. 对于悬浮细胞或细胞悬液：

- 用PBS或HBSS洗涤2次。
- 加入50 $\mu$ l TUNEL检测液，37 $^{\circ}$ C避光孵育60分钟。
- PBS或HBSS洗涤2次。
- 用250-500 $\mu$ l PBS或HBSS悬浮。
- 此时可以用流式细胞仪进行检测或涂片后在荧光显微镜下观察。可以使用的激发波长范围为450-500nm，发射波长范围为515-565nm(绿色荧光)。

#### 常见问题：

##### 1. 出现非特异性荧光标记。

- 有些细胞或组织，例如平滑肌细胞或组织，nuclease或polymerase的酶活性水平较高，易导致出现非特异性的荧光标记。解决方法是，取细胞或组织后立即固定并且要充分固定，以阻止这些酶导致假阳性。
- 使用了不适当的固定液，例如一些酸性固定液，导致出现假阳性。建议采用推荐的固定液。
- TUNEL检测反应时间过长，或TUNEL检测反应过程中反应液渗漏，细胞或组织表面不能保持湿润，也可能出现非特异性荧光。注意控制反应时间，并确保TUNEL检测反应液能很好地覆盖样品。

##### 2. 荧光背景很高。

- 支原体污染。请使用支原体染色检测试剂盒检测是否为支原体污染。支原体染色检测试剂盒(C0296)可以向碧云天订购。
- 高速分裂和增殖的细胞，有时也会出现细胞核中的DNA断裂。
- TUNEL反应过强。可以用试剂盒提供的TdT酶稀释液稀释TdT酶2-5倍后再按照说明书操作。稀释后的TdT酶需当日使用。
- 红细胞中血红蛋白导致的自发荧光产生严重干扰。此时宜选择其它细胞凋亡检测试剂盒。

##### 3. 标记效率低。

- 使用乙醇或甲醇固定会导致标记的效率较低。
- 固定时间过长，导致交联程度过高。此时宜减少固定时间。
- 荧光淬灭。荧光在普通光照10分钟就会严重淬灭。解决方法是需注意避光操作。
- 碘化丙啶双染时，如果碘化丙啶染色过深会导致观察到的本试剂盒的TUNEL染色效果减弱。碘化丙啶可以接受fluorescein激发产生的荧光，从而起到淬灭作用。解决方法是用较低浓度的碘化丙啶染色，例如0.5 $\mu$ g/ml碘化丙啶。
- 贴壁细胞如果使用药物诱导凋亡，会发生凋亡细胞的贴壁性会减弱，所以建议在凋亡诱导结束后，用可以对多孔板进行离心的离心机1000g离心5分钟，然后再吸除培养基并用PBS洗涤。如果没有适合的离心机，请注意操作轻缓，防止发生凋亡的细胞在洗涤时洗去。后续整个操作也需要轻缓。

#### 相关产品：

产品编号	产品名称	包装
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
C1062	Annexin V-FITC细胞凋亡检测试剂盒	20次
C1063	Annexin V-FITC细胞凋亡检测试剂盒	50次

C1065	Annexin V-PE细胞凋亡检测试剂盒	20次
C1067	Annexin V-EGFP细胞凋亡检测试剂盒	20次
C1068	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
P0098	免疫染色固定液	100ml
P0097-100ml	免疫染色强力通透液	100ml
P0097-500ml	免疫染色强力通透液	500ml
P0126	抗荧光淬灭封片液	5ml
ST533	Proteinase K (20mg/ml)	1ml

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