

TUNEL细胞凋亡检测试剂盒(显色法)

产品编号	产品名称	包装
C1098	TUNEL细胞凋亡检测试剂盒(显色法)	50次

产品简介:

- 碧云天生产的显色法TUNEL细胞凋亡检测试剂盒(Colorimetric 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。对于待检测的细胞或组织样品, 经过生物素标记和后续的DAB显色等步骤, 即可在普通光学显微镜下观察到凋亡细胞。
- 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)的催化下加上生物素(Biotin)标记的dUTP(Biotin-dUTP), 随后和辣根过氧化物酶(HRP)标记的Streptavidin (Streptavidin-HRP)结合, 最后在HRP的催化下通过DAB显色来显示凋亡细胞, 从而可以通过普通光学显微镜检测到凋亡的细胞, 这就是TUNEL(TdT-mediated dUTP Nick-End Labeling)法检测细胞凋亡的原理。
- 本试剂盒有如下优点。(1) 高灵敏度: 背景染色极低, 阳性染色强, 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) 特异性好: 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2-3个小时即可完成。(4) 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。(5) 实测效果好: 参考图1。

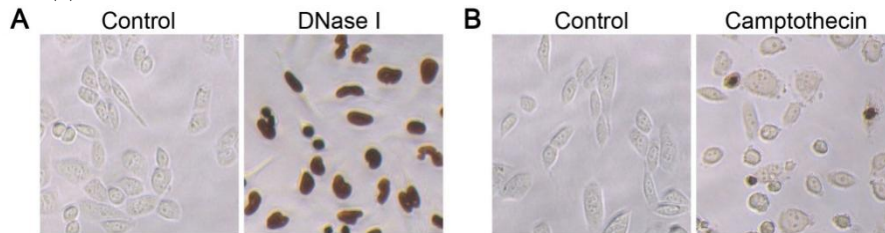


图1. 本试剂盒的检测效果图。A. HeLa细胞未处理或用DNase I室温孵育10分钟后的检测效果图。B. HeLa细胞未处理或用10 μ M喜树碱(Camptothecin)处理24小时后的检测效果图。图中棕褐色为DAB染色阳性细胞。本图中的染色实验均在12孔板中进行。本图仅作参考, 不同的样品不同的检测条件, 实际获得的结果可能和上图有较明显的差别。

- 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50个样品。

包装清单:

产品编号	产品名称	包装
C1098-1	TdT酶	250 μ l
C1098-2	Biotin-dUTP	2 \times 1.2ml
C1098-3	TdT酶稀释液(选用)	1ml
C1098-4	Streptavidin-HRP	55 μ l
C1098-5	Streptavidin-HRP稀释液	2 \times 1.25ml
C1098-6	DAB显色液A	15ml
C1098-7	DAB显色液B	15ml
C1098-8	标记反应终止液	15ml
—	说明书	1份

保存条件:

-20 $^{\circ}$ C保存, DAB显色液A和DAB显色液B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)等适当的封片液，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)。 需自备过氧化氢。
- 如果用于石蜡切片的检测，需自备蛋白酶K和二甲苯。蛋白酶K(ST533)可以向碧云天订购。
- DAB 对人体有害，操作时请小心，并注意有效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. 对于贴壁细胞或细胞涂片：

- a. 用PBS或HBSS洗涤1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4%多聚甲醛或碧云天生产的免疫染色固定液(P0098)固定细胞30分钟。
- d. 用PBS或HBSS洗涤1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. 用PBS或HBSS洗涤1次。
- g. 在碧云天生产的内源性过氧化物酶封闭液(P0100A)或PBS配制的0.3%过氧化氢溶液(0.3% H₂O₂ in PBS)中室温孵育20分钟，以灭活切片内源的过氧化物酶。随后用PBS或HBSS洗涤3次。
- h. 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)，用PBS或HBSS洗涤1次。
- b. 涂片，使细胞粘附在载玻片上。可以干燥样品使细胞贴得更牢，或使用适当的粘附试剂。
- 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1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. 用PBS或HBSS洗涤1次。
- g. 在碧云天生产的内源性过氧化物酶封闭液(P0100A)或PBS配制的0.3%过氧化氢溶液(0.3% H₂O₂ in PBS)中室温孵育20分钟，以灭活切片内源的过氧化物酶。随后用PBS或HBSS洗涤3次。
- h. 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20μg/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20μg/ml不含DNase的蛋白酶K)，20-37°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. 在碧云天生产的内源性过氧化物酶强力封闭液(P0100B)或PBS配制的3%过氧化氢溶液(3% H₂O₂ in PBS)中室温孵育20分钟，以灭活切片内源的过氧化物酶。随后用PBS或HBSS洗涤3次。注：请勿在用PBS配制的3%过氧化氢溶液中孵育过长时间，否则会出现过氧化氢导致的DNA断裂，从而产生假阳性。
- e. 转步骤5。

4. 对于冷冻切片：

- 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0.3% Triton X-100的PBS，室温孵育5分钟。
- d. 在碧云天生产的内源性过氧化物酶封闭液(P0100A)或PBS配制的0.3%过氧化氢溶液(0.3% H₂O₂ in PBS)中室温孵育20分钟，以灭活切片内源的过氧化物酶。随后用PBS或HBSS洗涤3次。
- e. 转步骤5。

5. 配制生物素标记液：

参考下表配制适量的生物素标记液，需充分混匀。注意：配制好的生物素标记液必须一次使用完毕，不宜冻存。

	1个样品	5个样品	10个样品
TdT酶	5μl	25μl	50μl
Biotin-dUTP	45μl	225μl	450μl
生物素标记液	50μl	250μl	500μl

6. 样品的生物素标记：

- a. 在样品上加50μl生物素标记液，37°C避光孵育60分钟。注意：50μl生物素标记液适合涂片、切片或96孔板、48孔板、24孔板或12孔板的一个孔，如果是6孔板中的一个孔生物素标记液宜使用100μl。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，滴加生物素

标记液后覆盖在样品上，可以防止生物素标记液蒸发，并且使生物素标记液均匀覆盖样品。自行裁剪圆片时需要连着圆片突出一个角或连着一一条，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，从而尽量减少生物素标记液的蒸发。

- b. 用PBS或HBSS洗涤1次，滴加0.1-0.3ml标记反应终止液，室温孵育10分钟。
- c. 用PBS或HBSS洗涤3次。

7. Streptavidin-HRP工作液和DAB显色液的配制：

- a. Streptavidin-HRP工作液的配制：

参考下表配制适量的Streptavidin-HRP工作液，需充分混匀。注意：配制好的Streptavidin-HRP工作液必须一次使用完毕，不宜冻存。

	1个样品	5个样品	10个样品
Streptavidin-HRP	1 μ l	5 μ l	10 μ l
Streptavidin-HRP稀释液	49 μ l	245 μ l	490 μ l
Streptavidin-HRP工作液	50 μ l	250 μ l	500 μ l

- b. DAB显色液的配制：

按照每个样品使用0.2-0.5ml显色液的比例配制适量DAB显色液。等体积混合适量DAB显色液A和DAB显色液B，充分混匀后即成为DAB显色液。注意：配制好的DAB显色液必须一次使用完毕，不宜冻存。

8. 样品的显色：

- a. 在样品上加50 μ l Streptavidin-HRP工作液，室温孵育30分钟。注意：50 μ l Streptavidin-HRP工作液适合涂片、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Streptavidin-HRP工作液宜使用100 μ l。如果待检测的样品为切片、涂片或在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Streptavidin-HRP工作液后覆盖在样品上，可以防止Streptavidin-HRP工作液蒸发，并且使Streptavidin-HRP工作液均匀覆盖样品。自行裁剪圆片时需要连着圆片突出一个角或连着一一条，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Streptavidin-HRP工作液的蒸发。
- b. 用PBS或HBSS洗涤3次。
- c. 滴加0.2-0.5ml DAB显色液，室温孵育5-30分钟或根据显色情况孵育适当时间。注：如果显色很强可以短于5分钟即停止显色，如果显色很弱，可以适当延长显色时间，甚至显色过夜。
- d. 用PBS或HBSS洗涤3次。
- e. 染色效果可参见图1或图2。
- f. 选做(本步骤可以不做)：用苏木素染色液(C0107)或甲基绿染色液(C0115)进行细胞核染色。随后用PBS或HBSS洗涤3次。
- g. 直接进行观察，或用95%乙醇脱水5分钟，再用100%乙醇脱水2次，每次约3分钟，再用二甲苯透明2次，每次5分钟，随后封片观察。染色结果可参考图2：

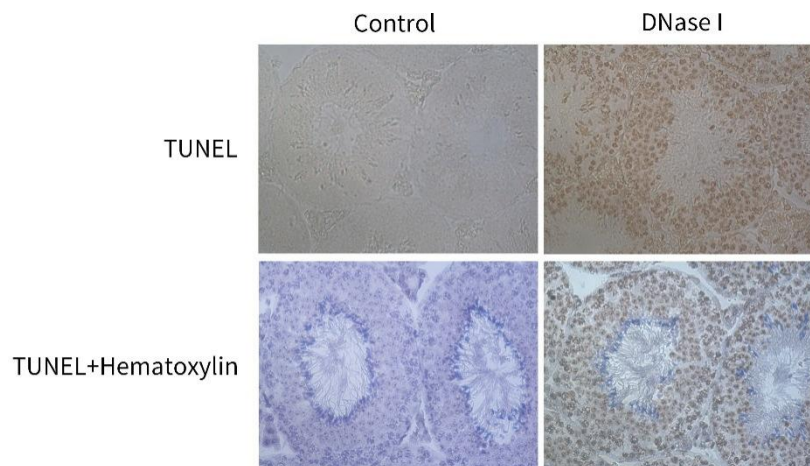


图2. TUNEL细胞凋亡检测试剂盒(显色法)用于小鼠睾丸切片的检测效果图。睾丸组织未处理(Control)或经DNase I处理60min后(DNase I)，用本试剂盒进行检测(TUNEL)，或本试剂盒检测后再用苏木素复染(TUNEL+Hematoxylin)。TUNEL染色阳性的凋亡细胞显棕色，苏木素染色的细胞核显蓝色，被TUNEL染色呈现阳性的细胞通常不能再被苏木素染色。实际检测效果会因样品和检测条件的不同而存在差异，本图仅作参考。

常见问题：

1. 出现非特异性显色。

- a. 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显色。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。
- b. 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。
- c. 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染色。

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. 染色背景很高。

- 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。
- 细胞内的过氧化物酶灭活不充分会导致很多细胞呈现阳性染色，改进过氧化物酶的灭活方法，例如延长灭活时间等会有所帮助。
- 所使用的溶液有DNA酶污染。DNA酶污染导致的随即切割会导致产生一定的背景并干扰检测。把溶液高压灭菌可以有效灭活各种常见DNA酶。

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。
- 贴壁细胞如果使用药物诱导凋亡，会使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，请注意操作轻柔，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柔。

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

使用本产品的文献：

- Zhang C, Wu R, Zhu H, Hu YZ, Jiang H, Lin NM, He QJ, Yang B. Enhanced anti-tumor activity by the combination of TRAIL/Apo-2L and combretastatin A-4 against human colon cancer cells via induction of apoptosis in vitro and in vivo. *Cancer Lett.* 2011 Mar 1;302(1):11-9.
- Xu X, Gao X, Jin L, Bhadury PS, Yuan K, Hu D, Song B, Yang S. Antiproliferation and cell apoptosis inducing bioactivities of constituents from *Dyosma versipellis* PC3 and Bcap-37 cell lines. *Cell Div.* 2011 Jun 15;6(1):14.
- Peng YR, Ding YF, Wei YJ, Shu B, Li YB, Liu XD. Caudatin-2,6-dideoxy-3-O-methyl-β-D-cymaropyranoside 1 induced apoptosis through caspase 3-dependent pathway in human hepatoma cell line SMMC7721. *Phytother Res.* 2011 May;25(5):631-7.
- Liu M, Yang S, Jin L, Hu D, Wu Z, Yang S. Chemical Constituents of the Ethyl Acetate Extract of *Belamcanda chinensis* (L.) DC Roots and Their Antitumor Activities. *Molecules.* 2012 May 24;17(5):6156-69.
- Liu MC, Yang SJ, Jin LH, Hu DY, Xue W, Song BA, Yang S. Synthesis and cytotoxicity of novel ursolic acid derivatives containing an acyl piperazine moiety. *Eur J Med Chem.* 2012 Dec;58:128-35.
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