一步法TUNEL细胞凋亡检测试剂盒
(TMR红色荧光)

产品编号

mlt69987

产品描述

细胞凋亡中染色体 DNA 的断裂是个渐进的阶段性过程。染色体 DNA 首先在内源性的核酸水解酶的作用下降解为 50-300 kb 的大片段，然后大约 30%的染色体 DNA 在 Ca2+和 Mg2+依赖的核酸内切酶作用下，在核小体单位之间被随机切断，形成 180-200 bp 核小体 DNA 多聚体。因此在细胞凋亡晚期，DNA 会被降解为 180-200 bp 的片段，断裂的基因组 DNA 上暴露出大量的 3'-OH 末端。末端脱氧核糖核苷酸转移酶（Terminal Deoxynucleotidyl Transferase, TdT）是一种不依赖于模板的 DNA 聚合酶，可以催化脱氧核苷酸结合到断裂的 DNA 分子 3'-OH 末端。因此 TUNEL (TdT mediated dUTP Nick End Labeling)细胞凋亡检测试剂盒可以用来检测组织细胞在凋亡晚期过程中细胞核 DNA 的断裂情况。其原理是在 TdT 酶的作用下，在基因组 DNA 断裂时暴露出的 3´-OH 末端掺入四甲基罗丹明-dUTP（Tetramethyl-Rhodamine-5-dUTP，TMR-5-dUTP），从而可以用荧光显微镜或流式细胞仪检测（TMR 激发 551 nm，发射 575 nm）。本试剂盒应用范围广，适用于石蜡组织切片，冰冻组织切片、细胞爬片、细胞涂片等的细胞凋亡检测。

储存与运输

本试剂盒储存在-20℃，TMR-5-dUTP Labeling Mix 需避光储存于-20℃，有效期 12 个月。

组成

产品名称	产品包装
Recombinant TdT Enzyme	45μL
TMR-5-dUTP Labeling Mix	220μL
Equilibration Buffer	5mL
Proteinase K（200 μg/mL）	500μL

实验前准备

1. PBS 磷酸盐缓冲液

2. 固定液：溶于 PBS 的 4%多聚甲醛，pH 7.4

3. 破膜液：0.1%-0.5% Triton X-100

4. 如需染核，需自备 DAPI（2 μg/mL）

5. 如需阳性对照实验，需自备 DNase I

6. 如果用流式细胞仪，自备 PI 染液和 RNase A（DNase free）

7. 操作时请穿实验服，佩戴一次性手套。

操作步骤

一、样品准备

A. 石蜡包埋组织切片

1. 室温下将石蜡组织切片放入环保型脱蜡透明液中浸泡 5-10 min，重复 3 次；然后无水乙醇浸泡 5 min，重复 2 次；最后用梯度乙醇（85%、75%）、双蒸水各浸泡 1 次，每次 5 min；

2. 用 PBS 轻轻润洗切片，并去掉样本周围多余液体；使用组化笔沿组织外围轮廓画一个与组织间隔 2-3 mm 的小圈，便于下游通透性处理和平衡标记操作；在实验过程中，切勿让样品干燥，处理好的样本放在湿盒中保持样本的湿润；

3. 配制 Proteinase K 工作液：按 1：9 体积比，用 PBS 作为稀释液来稀释 Proteinase K（200 μg/mL）原液，使其终浓度为 20 μg/mL；

4. 每个样本上滴加 100 μL 上述 Proteinase K 工作液，完全覆盖组织，37℃孵育 20 min；（注：Proteinase K 处理主要有助于组织和细胞后续步骤的染色试剂通透，其孵育时间过长过短都会影响后续标记效率，为得到更好的结果，可以优化 Proteinase K 孵育的时间）

5. 用 PBS 溶液浸润清洗样本 3 次，每次 5 min（Proteinase K 需洗涤干净，否则会干扰后续的标记反应）。处理后的样本放在湿盒中保持样本的湿润；

6. （可选步骤）去掉样本上多余的液体，将适量破膜液滴加到组织上，充分浸润组织，室温处理 20 min；破膜处理完成后同样的用 PBS 溶液润洗样本 3 次，每次 5 min；处理后的样本放在湿盒中保持样本的湿润。

B. 组织冰冻切片

1. 将玻片浸没在 4%多聚甲醛溶液（溶于 PBS）中固定，室温下孵育 10-15 min；

2. 片子从固定液中取出后，通风橱中自然晾干；

3. 将玻片放入纯水或 PBS 中润洗，去掉玻片上残存的固定液；

4. 用组化笔沿着组织外围轮廓画一个与组织间隔 2-3 mm 的小圈，便于下游通透性处理和平衡标记操作；在实验过程中，切勿让样品干燥，处理好的样本放在湿盒中保持样本的湿润；

5. 配制 Proteinase K 工作液：按 1：9 的比例，用 PBS 作为稀释液来稀释 Proteinase K（200 μg/mL）原液，使其终浓度为 20 μg/mL；

6. 每个样本上滴加 100 μL 上述 Proteinase K 工作液，使其被全部覆盖，室温孵育 10 min；（注：Proteinase K 处理主要有助于组织和细胞后续步骤的染色试剂通透。其孵育时间过长过短都会影响后续标记效率，未得到更好的结果，可能需要优化 Proteinase K 孵育的时

间)

7. 用 PBS 溶液润洗样本 2-3 次，去掉多余液体（Proteinase K 需洗涤干净，否则会干扰后续的标记反应），处理后的样本放在湿盒中保持样本的湿润；

8. （可选步骤）将适量破膜液滴加到组织上，充分浸润组织，室温处理 20 min，破膜处理完成后同样的用 PBS 溶液润洗样本，去掉多余液体，处理后的样本放在湿盒中保持样本的湿润。

C. 细胞爬片

1. 在共聚焦皿中培养贴壁细胞，在凋亡诱导处理之后，用 PBS 轻轻润洗 2 遍细胞；

2. 向每个小皿中加入适量的 4%多聚甲醛溶液（溶于 PBS）固定，室温下孵育 20 min；

3. 去掉固定液，加入 PBS 清洗 3 次，每次 5 min；

4. 每个样本浸于破膜液中，室温孵育 5 min 进行通透处理（注意：推荐用 2-20 μg/mL 的 Proteinase K 工作液消化，37℃处理 10 min 左右，视细胞状态调整。若细胞易掉片则建议选择用破膜液处理）；

5. 在盛有 PBS 溶液的敞口烧杯中浸没清洗样本 2-3 次；

6. 轻轻去掉多余液体，并用滤纸小心吸干载玻片上样本周围的液体。处理后的样本放在湿盒中保持样本的湿润。

D. 细胞涂片

1. 以约 2×107 个细胞/mL 的浓度将细胞重悬于 PBS 中，吸取 50-100 μL 细胞悬液滴于防脱玻片上，使用一片洁净的载玻片轻柔涂开细胞悬液；

2. 将玻片浸入装有 4%新鲜配制于 PBS 中的多聚甲醛的染色缸中，固定细胞，在 4℃放置 25 min；

3. 将玻片浸入 PBS 中，室温放置 5 min 浸洗，重复一次；

4. 轻轻去掉多余液体，并用滤纸小心吸干玻片上样本周围多余的液体，用组化笔沿着细胞外围轮廓画一个小圈，便于下游透性处理和平衡标记操作，在实验过程中，切勿让样品干燥；

5. 每个样本浸于破膜液中，室温孵育 5 min 进行通透处理（注意：推荐用 2-20 μg/mL 的 Proteinase K 工作液消化，37℃处理 10 min 左右，视细胞状态调整。若细胞易掉片则建议选择用破膜液处理）；

6. 在盛有 PBS 溶液的敞口烧杯中浸没清洗样本 2-3 次；

7. 轻轻去掉多余液体，并用滤纸小心吸干载玻片上样本周围的液体，处理后的样本放在湿盒中保持样本的湿润。

二、DNase I 处理阳性对照实验（可选步骤）

在样本通透处理后，用 DNase I 处理样本来准备阳性对照。

1. 将 100 μL 1×DNase I Buffer（配制方法：取 10 μL 10×DNase I Buffer，然后加入 90 μL 去离子水混匀）滴加到已通透的样本上，室温孵育 5 min；

2. 轻轻去掉多余液体，加入 100 μL 含有 DNase I (20 U/mL) 的工作液，室温孵育 10 min；

3. 轻轻去掉多余的液体，并将载玻片在装有 PBS 的染色缸中彻底洗 3-4 次。

三、标记与检测

1. 平衡：每个样本滴加 100 μL Equilibration Buffer 使其全部覆盖待检样本区域，室温孵育 10 min；

2. 标记液配制：在冰上解冻 TMR-5-dUTP Labeling Mix 和 Equilibration Buffer，并按照 Recombinant TdT enzyme：TMR-5-dUTP Labeling Mix：Equilibration Buffer=2 μL：10μL：100 μL（2：10：100）比例混合足够用于所有实验的 TdT 孵育缓冲液，具体实验

使用试剂的体积可以根据玻片的大小进行适当等比例调整；

3. 阴性对照体系：准备一份不含 Recombinant TdT enzyme 的对照 TdT 孵育缓冲液，用 ddH2O 替代；

4. 标记：尽量去掉平衡的 Equilibration Buffer，然后在每份组织样本上加入 112 μL TdT 孵育缓冲液，在 37℃孵育 1 h；注意不能干片，载玻片要避光；

5. 立即用 PBS 润洗组织样本，清洗 3 次，每次 5 min；

6. 用滤纸轻轻擦掉样本周围的 PBS 溶液；

7. 核染色：样本在染色缸中染色，在黑暗中将载玻片浸入装有 DAPI 溶液（用 PBS 新鲜配制并稀释）的染色缸，室温放置 8 min；

8. 封片：样本染色完成后，用 PBS 清洗组织样本 3 次，每次 5 min，然后轻轻去掉多余液体，滴加抗荧光淬灭封片剂封片；

9. 镜检：立即在荧光显微镜下分析样本，载玻片注意避光，DAPI 能将凋亡和未凋亡的细胞都染成蓝色，只在凋亡的细胞核中才有 TMR-5-dUTP 掺入而定位的红色荧光。

四、利用流式细胞术检测悬浮细胞

1. 将待检测细胞用 PBS 清洗两次，4℃离心（500 g）然后重悬在 500 μL PBS 中；

2. 固定：向样品中加入 5 mL 1%用 PBS 配制的多聚甲醛溶液，固定细胞，冰上放置 20 min；

3. 细胞在 4℃，300 g 离心 10 min，去上清并用 5 mL PBS 重悬两次，最后用 500 μL PBS 重悬细胞；

4. 通透：向样品中加入 5 mL 冰上预冷的 70%乙醇，-20℃孵育 4 h，通透细胞；

（注：细胞也可用破膜液室温 5 min 进行通透）

5. 细胞用 300 g 离心 10 min 后用 5 mL PBS 重悬，再次离心后用 1 mL PBS 重悬；

6. 平衡：转移约 2×106 个细胞至 1.5 mL 的微量离心管，300 g 离心 10 min，去上清，并用 100 μL Equilibration Buffer 重悬，室温孵育 5 min；

7. 标记液配制：在冰上解冻 TMR-5-dUTP Labeling Mix 和 Equilibration Buffer，并按照 Recombinant TdT enzyme：TMR-5-dUTP Labeling Mix：Equilibration Buffer=2 μL：10μL：100 μL（2：10：100）比例混合足够用于所有实验和可选阳性对照反应的 TdT 孵育缓冲液；

8. 标记:细胞用 300 g 离心 10 min,去上清将沉淀重悬在 112 μL TdT 孵育缓冲液中,37℃孵育 1 h，避光。每隔 15 min 用微量移液器轻轻重悬细胞；

9. 反应完成后加入 1 mL 20 mM EDTA 终止反应，用微量移液器轻柔混匀；

10. 300 g 离心 10 min，去上清并将沉淀重悬在 1 mL 破膜液中，其中含 5 mg/mL BSA，重复洗 2 次；

11. 核染色：300 g 离心 10 min，去上清并将细胞沉淀重悬在 0.5 mL DAPI 溶液中，其中包含 250 μg 无 DNA 酶的 RNase A，在黑暗中室温孵育细胞 30 min；

12. 上机检测：流式细胞仪分析细胞，DAPI 能将凋亡和未凋亡的细胞核均染成蓝色，只在凋亡的细胞核中才有 TMR-5-dUTP 掺入而定位的红色荧光。

TMR (red) Tunel Cell Apoptosis Detection Kit

Product Description

Breakage of chromosomal DNA in apoptosis is a gradual phased process. Chromosomal DNA is first degraded into large fragments of 50-300 kb by endogenous nucleases, and then about 30% of chromosomal DNA is randomly cleaved between nucleosome units under the action of Ca2 and Mg2-dependent

endonucleases to form 180-200 bp nucleosome DNA multimers. Therefore, in the late apoptosis stage, DNA is degraded into 180-200 bp fragments, exposing a large number of 3'-OH terminations on the broken genomic DNA. Terminal deoxynucleotidyl transferase (TdT) is a template-independent DNA polymerase that catalyzes deoxynucleotide binding to the 3'-OH end of a broken DNA molecule. Therefore, the TUNEL (TdT mediated dUTP Nick End Labeling) apoptosis detection kit can be used to detect the breakage of nuclear DNA in tissue cells during the advanced apoptosis process. The principle is that the 3 -OH end exposed at the time of genomic DNA breakage is incorporated with tetramethyl-rhodamine-dUTP (TMR-5-dUTP, TMR-5-dUTP) under the action of the TdT enzyme, which can be detected by fluorescence microscopy or flow cytometry (TMR excitation 551 nm, emission 575 nm). This kit has a wide range of applications, and is suitable for apoptosis detection of paraffin tissue sections, frozen tissue sections, cell crawling sections, cell smears, etc.

Storage and transportation

This kit is stored at -20°C, and TMR-5-dUTP Labeling Mix needs to be stored at -20° C in a dark place for 12 months.

Preparation Before the Experiment

1. PBS (Phosphate-Buffered Saline)

2. Fixative: 4% paraformaldehyde dissolved in PBS, pH 7.4

3. Permeabilization solution: 0.1%-0.5% Triton X-100

4. If nuclear staining is needed, prepare DAPI (2 μg/mL) yourself

5. If performing a positive control experiment, prepare DNase I yourself

6. If using a flow cytometer, prepare PI staining solution and RNase A (DNase free) yourself

7. Wear a lab coat and disposable gloves during operation.

Procedure:

1. Sample preparation

A. Paraffin-embedded tissue sections

1. Soak the paraffin tissue sections in environmentally friendly dewaxing transparent solution for 5-10 minutes at room temperature and repeat 3 times. Then soak in anhydrous ethanol for 5 minutes and repeat twice; Finally, soak in gradient ethanol (85% and 75%) and double distilled water once for 5 minutes each time.

2. Gently wash the sections with PBS and remove excess liquid around the sample. Use a grouping pen to draw a small circle with a distance of 2-3 mm from the tissue along the outer contour of the tissue to facilitate downstream permeability processing and balance marking operation. During the experiment, do not let the sample dry, and keep the sample moist in the wet box.

3. Preparation of Proteinase K working solution: According to the volume ratio of 1:9, PBS was used as a dilution to dilute Proteinase K (200 μg/mL) stock solution to make the final concentration 20 μg/mL.

4. Add 100 μL of the above Proteinase K working solution to each sample, completely cover the tissue, and incubate at 37°C for 20 min;

(Note: Proteinase K treatment mainly helps the staining reagent to be permeable in the next steps of tissues and cells, and its incubation time will affect the subsequent labeling efficiency if it is too long or too short, so the incubation time of Proteinase K can be optimized for better results)

5. Wash the samples with PBS solution infiltrate 3 times for 5 min each time (Proteinase K needs to be washed clean, otherwise it will interfere with subsequent labeling reactions). The treated samples are placed in a wet box to keep the samples moist;

6. (Optional step) Remove the excess liquid from the sample, add an appropriate amount of membrane rupture droplets to the tissue, fully infiltrate the tissue, and treat at room temperature for 20 minutes. After the film breaking treatment was completed, the samples were washed with PBS solution 3 times for 5 minutes each time. The treated sample is placed in a wet box to keep the sample moist.

B. Organizing Frozen Sections

1. Immerse the slides in 4% paraformaldehyde solution (dissolved in PBS) for fixation and incubate at room temperature for 10-15 minutes;

2. Remove the slides from the fixative and air dry them naturally in a fume hood;

3. Rinse the slides in pure water or PBS to remove any residual fixative on the slides;

4. Use a tissue-marking pen to draw a small circle around the tissue outline, leaving

a 2-3 mm gap from the tissue, to facilitate downstream permeability treatment and balanced labeling operations; during the experiment, do not let the samples dry, and keep treated samples in a humid box to maintain moisture;

5. Prepare Proteinase K working solution: dilute the Proteinase K stock solution (200 μg/mL) with PBS at a ratio of 1:9 to reach a final concentration of 20 μg/mL;

6. Add 100 μL of the Proteinase K working solution to each sample, ensuring complete coverage, and incubate at room temperature for 10 minutes;

(Note: Proteinase K treatment mainly helps improve the permeability of tissues and cells for subsequent staining reagents. Incubation time that is too long or too short may affect the efficiency of downstream labeling. If optimal results are not achieved, the Proteinase K incubation time may need to be optimized.)

7. Rinse the samples 2-3 times with PBS to remove excess liquid (Proteinase K must be thoroughly washed off, otherwise it will interfere with subsequent labeling reactions). Keep the treated samples in a humid box to maintain moisture;

8. (Optional) Add an appropriate amount of permeabilization solution to the tissue, fully infiltrate the tissue, and treat at room temperature for 20 minutes. After permeabilization, rinse the samples with PBS to remove excess liquid, and keep the treated samples in a humid box to maintain moisture.

C. Cell Spreading on Coverslips

1. Culture adherent cells in confocal dishes. After apoptosis induction treatment, gently rinse the cells twice with PBS;

2. Add an appropriate amount of 4% paraformaldehyde solution (dissolved in PBS) to each small dish for fixation and incubate at room temperature for 20 minutes;

3. Remove the fixative and wash three times with PBS, 5 minutes each time;

4. Immerse each sample in permeabilization solution and incubate at room temperature for 5 minutes for permeabilization (Note: It is recommended to digest with 2-20 μg/mL Proteinase K working solution at 37°C for about 10 minutes, adjusting as needed based on cell condition. If cells are prone to detachment, using permeabilization solution is recommended);

5. Immerse the samples in an open beaker containing PBS solution and rinse 2-3 times;

6. Gently remove excess liquid and carefully blot the surroundings of the samples on the slides with filter paper. Place the treated samples in a humidified box to keep them moist.

D. Cell Smear

1. Resuspend the cells in PBS at a concentration of approximately 2×10^7 cells/mL. Pipette 50-100 μL of the cell suspension onto an anti-fall glass slide, and gently spread the cell suspension using a clean coverslip.

2. Immerse the slide in a staining jar containing 4% freshly prepared paraformaldehyde in PBS to fix the cells, and leave it at 4°C for 25 minutes.

3. Immerse the slide in PBS and let it wash at room temperature for 5 minutes, then repeat once.

4. Gently remove the excess liquid and carefully absorb any remaining liquid around the sample on the slide with filter paper. Draw a small circle around the cell perimeter with a marker to facilitate subsequent permeability treatment and labeling procedures. During the experiment, do not let the sample dry.

5. Immerse each sample in permeabilization solution and incubate at room temperature for 5 minutes for permeabilization (Note: It is recommended to digest with Proteinase K working solution at 2-20 μg/mL, treating at 37°C for about 10 minutes, adjusting according to the cell condition. If cells tend to detach, it is recommended to use permeabilization solution instead).

6. Immerse and wash the samples 2-3 times in an open beaker containing PBS solution.

7. Gently remove the excess liquid and carefully absorb any remaining liquid around the sample on the slide with filter paper. Place the treated samples in a humidified chamber to keep them moist.

2. DNase I Treatment Positive Control Experiment (Optional Step)

After sample permeabilization, treat the samples with DNase I to prepare a positive control.

1. Add 100 μL of 1× DNase I Buffer (prepared by mixing 10 μL of 10× DNase I Buffer with 90 μL of deionized water) to the permeabilized samples and incubate at room temperature for 5 minutes;

2. Gently remove the excess liquid, add 100 μL of the working solution containing

DNase I (20 U/mL), and incubate at room temperature for 10 minutes;

3. Gently remove the excess liquid and thoroughly wash the slides 3–4 times in a staining jar containing PBS.

3. Labeling and Detection

1. Equilibration: Add 100 μL of Equilibration Buffer to each sample to completely cover the area to be tested, and incubate at room temperature for 10 minutes.

2. Labeling solution preparation: Thaw TMR-5-dUTP Labeling Mix and Equilibration Buffer on ice, and mix them according to the ratio Recombinant TdT enzyme:TMR-5-dUTP Labeling Mix:Equilibration Buffer = 2 μL:10 μL:100 μL (2:10:100) to make enough TdT incubation buffer for all experiments. The volume of reagents used in specific experiments can be appropriately adjusted proportionally based on the size of the slides.

3. Negative control system: Prepare a control TdT incubation buffer without Recombinant TdT enzyme, replacing it with ddH2O.

4. Labeling: Remove as much of the equilibrated Equilibration Buffer as possible, then add 112 μL of TdT incubation buffer to each tissue sample and incubate at 37° C for 1 hour. Ensure the slides do not dry out and keep them away from light.

5. Immediately wash the tissue samples with PBS three times, 5 minutes each wash.

6. Gently wipe off PBS around the samples with filter paper.

7. Nuclear staining: Place the samples in a staining jar, immerse the slides in a jar containing DAPI solution (freshly prepared and diluted with PBS) in the dark, and

let stand at room temperature for 8 minutes.

8. Mounting: After staining, wash the tissue samples with PBS three times, 5 minutes each time, gently remove excess liquid, and add anti-fade mounting medium for mounting.

9. Microscopy: Analyze the samples immediately under a fluorescence microscope, keeping the slides away from light. DAPI stains both apoptotic and non-apoptotic cells blue, while the red fluorescence from TMR-5-dUTP incorporation is only localized in the nuclei of apoptotic cells.

4. Detection of suspended cells by flow cytometry

1. Wash the cells to be tested twice with PBS, centrifuge at 4 °C (500 g) and resuspend in 500 μL PBS;

2. Fixation: Add 5 mL of 1% paraformaldehyde solution prepared with PBS to the sample, fix the cells, and place on ice for 20 minutes;

3. Cells were centrifuged at 300 g at 4 °C for 10 min, desupernatant and resuspended twice with 5 mL PBS, and finally resuspended with 500 μL PBS.

4. Permeabilization: Add 5 mL of pre-chilled 70% ethanol on ice to the sample, incubate at -20°C for 4 h, permeate the cells;

(Note: Cells can also be permeated by membrane breaking solution at room temperature for 5 minutes)

5. Cells were centrifuged with 300 g for 10 min and resuspended with 5 mL PBS, and re-centrifuged with 1 mL PBS.

6. Equilibration: Transfer approximately 2×106 cells to 1.5 mL microcentrifuge tubes, centrifuge 300 g for 10 min, remove the supernatant, and resuspend with 100 μL Equilibration Buffer and incubate for 5 min at room temperature.

7. Labeling solution preparation: Thaw TMR-5-dUTP Labeling Mix and Equilibration Buffer on ice and follow Recombinant TdT enzyme: TMR-5-dUTP Labeling Mix: Equilibration Buffer=2 μL: 10 μL: 100 μL (2:10:100) ratio mix enough TdT incubation buffer for all experiments and optional positive control reactions;

8. Labeling: Cells were centrifuged with 300 g for 10 min, desupernatant and resuspended in 112 μL TdT incubation buffer and incubated at 37 °C for 1 h away from light. Gently resuspend cells with a micropipette every 15 min;

9. After the reaction is completed, add 1 mL of 20 mM EDTA to terminate the reaction and gently mix well with a micropipette.

10. Centrifuge 300 g for 10 min, remove the supernatant and resuspend the pellet in 1 mL of membrane breaking solution containing 5 mg/mL BSA, repeat the wash twice;

11. Nuclear staining: 300 g centrifuge for 10 min, desupernatant and resuspend the cell pellet in 0.5 mL DAPI solution containing 250 μg DNase-free RNase A, incubate cells at room temperature in the dark for 30 min;

12. On-machine detection: Flow cytometry analyzes cells, DAPI can stain both apoptotic and non-apoptotic nuclei in blue, and only the apoptotic nuclei have red fluorescence localized by TMR-5-dUTP incorporation.