

Product Data Sheet

PBS, ddH₂O, Antifade Mounting Medium with DAPI (Cat.No. GC26283)

II 实验

1. 实验步骤

1.1. 实验步骤

1.1.1. 将细胞爬片放入 TC 培养皿中，加入 PBS 200 μL

1.1.2. 加入 4% 多聚甲醛 4°C 固定 25 min

1.1.3. 加入 PBS 2-3 次，每次 5 min

1.1.4. 加入 0.2% Triton X-100 5 min

注意：加入 Triton X-100 后加入 Proteinase K 1:100 加入 PBS 2 mg/mL Proteinase K 20 μg/mL 100 μL Proteinase K 5 min 15 min

1.1.5. 加入 PBS 2-3 次，每次 5 min

1.2. 实验步骤

1.2.1. 加入 ddH₂O 10×DNase I Buffer

1.2.2. 加入 1:10 ddH₂O 10×DNase I Buffer 1×DNase I Buffer

1.2.3. 加入 100 μL 1×DNase I Buffer 5 min

1.2.4. 加入 1×DNase I Buffer DNase I (2000 U/mL) 20 U/mL

1.2.5. 加入 50 μL 20 U/mL DNase I

1.2.6. 加入 20 U/mL DNase I 37°C 10 min

1.2.7. 加入 PBS 3 次，每次 5 min

注意：加入 DNase I 后加入 PBS 3 次，每次 5 min

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1.3. 试剂盒

1.3.1. 试剂盒组成

1.3.2. 1:5 ddH₂O 5×Equilibration Buffer 1×Equilibration Buffer

1.3.3. 100μL 1×Equilibration Buffer 1×Equilibration Buffer 5-10min

1.3.4. 试剂盒组成

ddH₂O 35μL 34μL

5×Equilibration Buffer 10μL 10μL

CY3-dUTP Mix 5μL 5μL

TdT Enzyme 0 1μL

1.3.5. 试剂盒组成

5cm² 50μL 50μL TdT

1.3.6. 37°C 60min

1.3.7. PBS 2 5min

PBS 0.1% Triton X-100 5mg/mL BSA PBS 3 5min

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1.3.8 Antifade Mounting Medium with DAPI (Cat.No. GC26283)

1.3.9 Antifade Mounting Medium with DAPI

1.3.10 550/570nm; 356/451nm DAPI

2. 2.1.1.

2.1. 2.1.1.

2.1.1. 2×10^6 cells/mL PBS 50-100 μ L

2.1.2. 4% 4°C 25min

2.1.3. PBS 2-3 5min

2.1.4. 0.2% Triton X-100 5min

Triton X-100 Proteinase K 1:100 PBS 2mg/mL Proteinase K 20 μ g/mL 100 μ L Proteinase K 5min

2.1.5. PBS 2-3 5min

2.1.6.

2.2. 2.2.1.

2.2.1. 1:10 ddH₂O 10×DNase I Buffer 1×DNase I Buffer

2.2.2. 1:10 ddH₂O 10×DNase I Buffer 1×DNase I Buffer

2.2.3. 100 μ L 1×DNase I Buffer 5min

2.2.4. 1×DNase I Buffer DNase I(2000U/mL) 20U/mL

2.2.5. 100 μ L 20U/mL DNase I

2.2.6. 37°C 10min

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2.2.7. 加入PBS 3 5min

注: DNase I

2.3. 步骤

2.3.1. 步骤

2.3.2. 1:5 ddH₂O 5×Equilibration Buffer 1×Equilibration Buffer

2.3.3. 100μL 1×Equilibration Buffer 5-10min

2.3.4. 步骤

加入 加入 + 加入

ddH₂O 35μL 34μL

5×Equilibration Buffer 10μL 10μL

CY3-dUTP Mix 5μL 5μL

TdT Enzyme 0 1μL

2.3.5. 50μL

注: 5cm² 50μL 50μL TdT

2.3.6. 37°C 60min

2.3.7. PBS 2 5min

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步骤: 用 PBS 清洗 0.1% Triton X-100 5mg/ml BSA PBS 3 5min

2.3.8 Antifade Mounting Medium with DAPI (Cat.No. GC26283)

2.3.9 Antifade Mounting Medium with DAPI

2.3.10 550/570nm; 356/451nm DAPI

3. 步骤

3.1. 步骤

3.1.1. 2 5-10min

20°C 20min

3.1.2. 2 5min

3.1.3. 90% 80% 70% 1 3min

3.1.4. PBS

3.1.5 1:100 PBS 2mg/mL Proteinase K 20µg/mL 100µL Proteinase K 37°C 15-30

步骤: Proteinase K

3.1.6. PBS 2-3 5min

步骤: Proteinase K

3.2. 3.3 2.2 2.3

4. 步骤

4.1. 步骤

4.1.1. 20min

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4.1.2. 加入4%的福尔马林固定30min

4.1.3. 用PBS清洗3次

4.1.4. 用PBS清洗2次5min

4.1.5. 加入100μL 0.2% Triton X-100 清洗15-30min
1:100 PBS 2mg/mL Proteinase K 20μg/mL 100μL
Proteinase K 37°C 10-30min

注意: Proteinase K 处理时需在37°C 避光进行

4.1.6. 用 PBS 清洗2-3次5min

注意: Proteinase K 处理时需在37°C 避光进行

4.2. 4.3 2.2 2.3

III 步骤

1. 5×Equilibration Buffer / PAP Pen

2. 5×Equilibration Buffer

3. CY3-dUTP Mix TdT Enzyme CY3-dUTP Mix TdT Enzyme -20°C

4. DNase I

5.

6.

7.

8.

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Proteinase K 2mg/mL 100μL

0.2% Triton X-100 1~2μL

DNase I 2U/μL 10x DNase I Buffer

1. Proteinase K 2mg/mL 100μL
2. DNase I 2U/μL 10x DNase I Buffer

Background

GlpBio One-step TUNEL Apoptosis Detection Kit (Orange, CY3) DNA Ex/Em = 550/570nm

DNA 180-200bp 3'-OH DNA 3'-OH TdT dUTP DNA 3'-OH

1. Proteinase K (2mg/mL)

2. DNase I (2U/μL)

1. Proteinase K (2mg/mL)
2. DNase I (2U/μL) 10x DNase I Buffer
3. 5x Equilibration Buffer CY3-dUTP Mix TdT Enzyme

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