TUNEL In Situ Cell Apoptosis Detection Kit

Catalog No.	Dye
abx092371	FITC
abx092372	AF488
abx092373	AF594
abx092374	AF647
abx092375	AF555

Size: 50 tests / 100 tests

Storage: Store all components in the dark at -20 °C. Avoid prepeated freeze/thaw cycles.

Application: For detecting cell apoptosis in tissue (paraffin-embedded or frozen) and cells (cell slides and cell smears).

Introduction

During apoptosis, specific DNA endonucleases are activated, cleaving genomic DNA between nucleosomes. The DNA of apoptotic cells is cleaved into multimers of 180-200 bp fragments. Fluorescent-labeled nucleotides can be attached to the fragments via the exposed 3'-OH end of the cleaved DNA, catalyzed by terminal deoxynucleotidyl transferase, which can be analyzed using a fluorescence microscope.

Kit Components (50 tests)

1. TdT Equilibration Buffer 9 ml
2. TdT Enzyme: 250 µl
3. Proteinase K (100X): 50 µl
4. Labelling Solution (see table above): 5 × 100 µl
5. DNase I (20 U/µl): 13 µl
6. DNase I Buffer (10X): 700 µl
7. DAPI Reagent (25 µg/ml): 250 µl

Kit Components (100 tests)

1. TdT Equilibration Buffer 2 × 9 ml
2. TdT Enzyme: 2 × 250 µl
3. Proteinase K (100X): 100 µl
4. Labelling Solution (see table above): 10 × 100 µl
5. DNase I (20 U/µl): 25 µl
6. DNase I Buffer (10X): 1500 µl
7. DAPI Reagent (25 µg/ml): 500 µl

Materials Required But Not Provided

1. Fluorescence microscope
2. PBS
3. Double-distilled water
4. Anti-fluorescence quenching reagent
5. Centrifuge and centrifuge tubes
6. Pipettes and pipette tips
7. Filter paper
8. Polyformaldehyde
9. Triton-100
10. Xylene
11. Ethanol

Protocol

A. Reagent Preparation

1. Proteinase K Working Solution

Dilute the 100X Proteinase K 100-fold with PBS to prepare the 1X Proteinase K Working Solution (e.g. add 1 µl of 100X Proteinase K to 99 µl of PBS to prepare 100 µl of 1X Proteinase K Working Solution). Mix well. Prepare only prior to immediate use.

2. DNase I Buffer Solution

Dilute the 10X DNase I Buffer 10-fold with double-distilled water to prepare the 1X DNase I Buffer Solution. Mix well. Prepare only prior to immediate use.

3. DNase I Working Solution

Dilute the DNase I (20 U/µl = 20,000 U/ml) 100-fold with 1X DNase I Buffer Solution to prepare the DNase I Working Solution (0.2 U/µl = 200 U/ml). Mix well. Prepare only prior to immediate use. Do not vortex.

4. DAPI Reagent Working Solution

Dilute the DAPI Reagent 25-fold with PBS to prepare the DAPI Reagent Working Solution (e.g. add 4 µl of DAPI Reagent to 96 µl of PBS to prepare 100 µl of DAPI Reagent Working Solution). Mix well. Prepare only prior to immediate use.

5. Fixative Buffer

Dissolve polyformaldehyde in PBS to a final concentration of 4%.

6. Cell Permeabilization Buffer

Dissolve Triton-100 in PBS to a final concentration of 0.2%. Once prepared, the solution can be stored at 4 °C for up to 2 days.

7. Labelling Working Solution

For each sample slide and positive control slide, prepare 50 µl of Labelling Working Solution by mixing 35 µl of TdT Equilibration Buffer, 10 µl of Labelling Solution, and 5 µl of TdT Enzyme. Prepare only prior to immediate use.

8. Negative Control Labelling Working Solution

For each negative control slide, prepare 50 µl of Negative Control Labelling Working Solution by mixing 40 µl of TdT Equilibration Buffer and 10 µl of Labelling Solution. This solution does not contain TdT Enzyme. Prepare only prior to immediate use.

B. Sample Fixation and Permeabilization

- **Cell Samples**
  1. **Cell slides:** Wash slides in PBS. Use filter paper to absorb excess liquid around the sample. Immerse slides in Fixative Buffer at room temperature for 15-20 minutes, or at 4 °C for 1-2 hours.

     Cell smears: Collect cells and resuspend in PBS. Add an equal volume of Fixative Buffer to the volume of PBS previously added. Allow the mixture to stand at room temperature for 15-20 minutes, or at 4 °C for 1-2 hours. Centrifuge at 600 × g for 5 minutes. Discard the supernatant, then add PBS to resuspend the cells. On a slide, aliquot 25-50 µl of cell suspension and allow to dry.
  2. Wash slides with PBS 3 times, 5 minutes each time.
  3. Immerse slides in Cell Permeabilization Buffer and incubate at 37 °C for 10 minutes.
  4. Wash slides with PBS 3 times, 5 minutes each time.

- **Paraffin Sections**
  1. Deparaffinize and hydrate paraffin slides using conventional methods. Immerse slides in xylene 2 times, 10 minutes each time (may be increased to 20 minutes if the ambient temperature is lower than 20 °C). Hydrate paraffin sections with decreasing concentrations of ethanol (100%, 95%, 90%, 80%, 75%), 3 minutes each.
  2. Wash slides with PBS 3 times, 5 minutes each time.
  3. Use filter paper to absorb excess liquid around the sample. Add 100 µl of 1X Proteinase K Working Solution to each sample. Incubate at 37 °C for 20 minutes. The incubation time can be adjusted depending on the sample.
  4. Wash slides with PBS 3 times, 5 minutes each time.

- **Frozen Sections**
  1. Equilibrate frozen sections to room temperature, then immerse frozen slides in Fixative Buffer at room temperature for 15-20 minutes.
  2. Wash slides with PBS 2 times, 5 minutes each time.
  3. Add 100 µl of 1X Proteinase K Working Solution to each sample. Incubate at 37 °C for 10-20 minutes. The incubation time can be adjusted depending on the sample.
  4. Wash slides with PBS 3 times, 5 minutes each time.

C. Positive and Negative Control Preparation

It is recommended to select representative samples as positive and negative controls. Positive controls are treated with DNase I to produce exposed 3'-OH ends; and are used to verify the effectiveness of the experimental process and reagents. Negative controls are treated with a labelling solution that does not include TdT Enzyme; and are used to determine non-specific staining of samples and sample autofluorescence.

1. Add 100 µl of 1X DNase I Buffer to each positive control slide and negative control slide. Allow the slides to stand at room temperature for 5 minutes.
2. **Positive control slides:** Use filter paper to absorb excess liquid around the sample. Add 100 µl of DNase I Working Solution (200 U/ml) to each positive control slide, then incubate at 37 °C for 10-30 minutes.

   Negative control slides: Incubate at 37 °C for 10-30 minutes.
3. Wash slides with PBS 3 times, 5 minutes each time.

D. Labelling

Notes:

- Bring the TdT Equilibration Buffer to room temperature and mix well before use. Crystals may be observed when the TdT Equilibration Buffer is taken out of the freezer; they will dissolve at room temperature. Ensure all crystals are dissolved prior to use.
- Store the TdT Enzyme at -20 °C immediately after the labelling working solutions are prepared. Do not vortex the TdT Enzyme.
- Before using the labelling working solutions, keep on ice and mix well.

Labelling Protocol

1. Add 100 µl of TdT Equilibration Buffer to each sample. Incubate at 37 °C for 10-30 minutes.
2. Use filter paper to absorb excess liquid around the sample. Do not allow the samples to dry out completely.

3. **Sample slides and positive control slides:** Add 50 µl of Labelling Working Solution to each sample slide and positive control slide.

   Negative control slides: Add 50 µl of Negative Control Labelling Working Solution to each negative control slide.

4. Incubate at 37 °C for 1 hour in the dark in a humidified chamber. If the signal intensity is low, the incubation time can be extended up to 4 hours.

5. Wash slides with PBS 3 times, 5 minutes each time.

6. Use filter paper to absorb excess liquid around the sample. Add DAPI Reagent Working Solution and allow to stand in the dark for 5 minutes.

7. Use filter paper to absorb excess liquid around the sample. Add Anti-fluorescence quenching reagent to seal the slides.

E. Analysis

Samples can be analyzed using a fluorescence microscope with an appropriate filter. It is recommended to analyze samples as soon as possible or store samples at 4 °C in the dark.

Dye	Ex/Em (nm)	Filter Set
FITC	490/520	FITC Filter Set
AF488	495/519	FITC Filter Set
AF594	590/617	TRITC Filter Set
AF647	650/665	Cy5 Filter Set
AF555	555/565	TRITC Filter Set
DAPI	350/470	DAPI Filter Set

F. Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.