TUNEL Apoptosis Detection Kit **Cat. No. L00300**
(For Paraffin-embedded Tissue Sections, FITC-labeled POD)

Technical Manual No. 0268 **Version 01132011**

I. DESCRIPTION

The **TUNEL Apoptosis Detection Kit for Paraffin-embedded Tissue Sections** (FITC-labeled POD) (**Cat. No. L00300**) is one of GenScript's newly introduced products. The kit can detect fragmented DNA in the nucleus during apoptosis. In this modified TUNEL assay kit, fluorescein-labeled nucleotides bind with the DNA 3'-OH ends using natural or recombinant terminal deoxynucleotidyl transferase (TdT or rTdT). The fluorescence could be observed by fluorescence microscope. And then the compound of anti-fluorescein antibody and HRP is bound to these fluorescein labeled nucleotides, which are detected using the peroxidase substrate, hydrogen peroxide, and 3,3'-diaminobenzidine (DAB), a stable chromogen. Using this procedure, apoptotic nuclei are stained dark brown.

II. KEY FEATURES

- **Simplified Procedure:** The kit contains ready-to-use reagents, including proteinase K, DAB and DNase I.
- **Enhanced Sensitivity:** This kit can assay the cells during the early stages of apoptosis.
- **Enhanced Specificity:** The kit can stain apoptotic cells.
- **Streamlined Process:** The entire procedure takes about three hours.
- **Increased Convenience:** The results can be observed by fluorescence microscope and light microscope.
- **High Veracity：** The kit contains positive control reagent.

III. KIT CONTENTS

The TUNEL Apoptosis Detection Kit (L00300) is for detection using fluorescein Labeled nucleotides (FITC-12-dUTP), HRP-labeled anti-FITC antibody, Proteinase K, TdT and DAB.

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Components	Cat. No. L00300 20 Assays	Cat. No. L00300 50 Assays	Cat. No. L00300 100 Assays	Storage Conditions
Equilibration Buffer	1 ml	2.5 ml	5.0 ml	-20°C
FITC-12-dUTP	20 µl	50 µl	100 µl	-20°C
TdT	80 µl	200 µl	400 µl	-20°C
50X Proteinase K （1 mg/ml）	40 µl	100 µl	200 µl	-20°C
HRP-labeled Anti-FITC Antibody	200 µl	500 µl	1000 µl	-20°C
DAB	2 mg	5 mg	10 mg	-20°C
DNase I (50 U/µl)	0.2 ml	0.5 ml	1 ml	-20°C
1×DNase I buffer	0.2 ml	0.5 ml	1 ml	4°C

IV. STORAGE

Store DNase I buffer at 4°C. Store the rest of the kit at -20°C. It will remain stable for one year.

V. PROTOCOL

Specificition： For paraffin-embeded sections, the suitable thickness is about 5 µm, but no more than 10 µm. Only if the paraffin section is thicker than 10 µm, the kit may not work well.

Before use, order or prepare the following:

Fixation Solution: 4% paraformaldehyde in PBS, pH 7.4, freshly prepared.

Blocking Solution: 3% H_2O_2 in methanol.

Permeabilization Solution: 0.1% Triton X-100 and 0.1% sodium citrate in water, freshly prepared.

Note:

1. Please centrifuge the reagents in the kit before use.
2. Please prepare the proper amount of **TUNEL Reaction Mixture** according to the amount of the samples to save reagent.
3. The DAB is powder, please dissolve the DAB powder in PBS to make 20×DAB buffer (10 mg/ml DBA buffer) before use.

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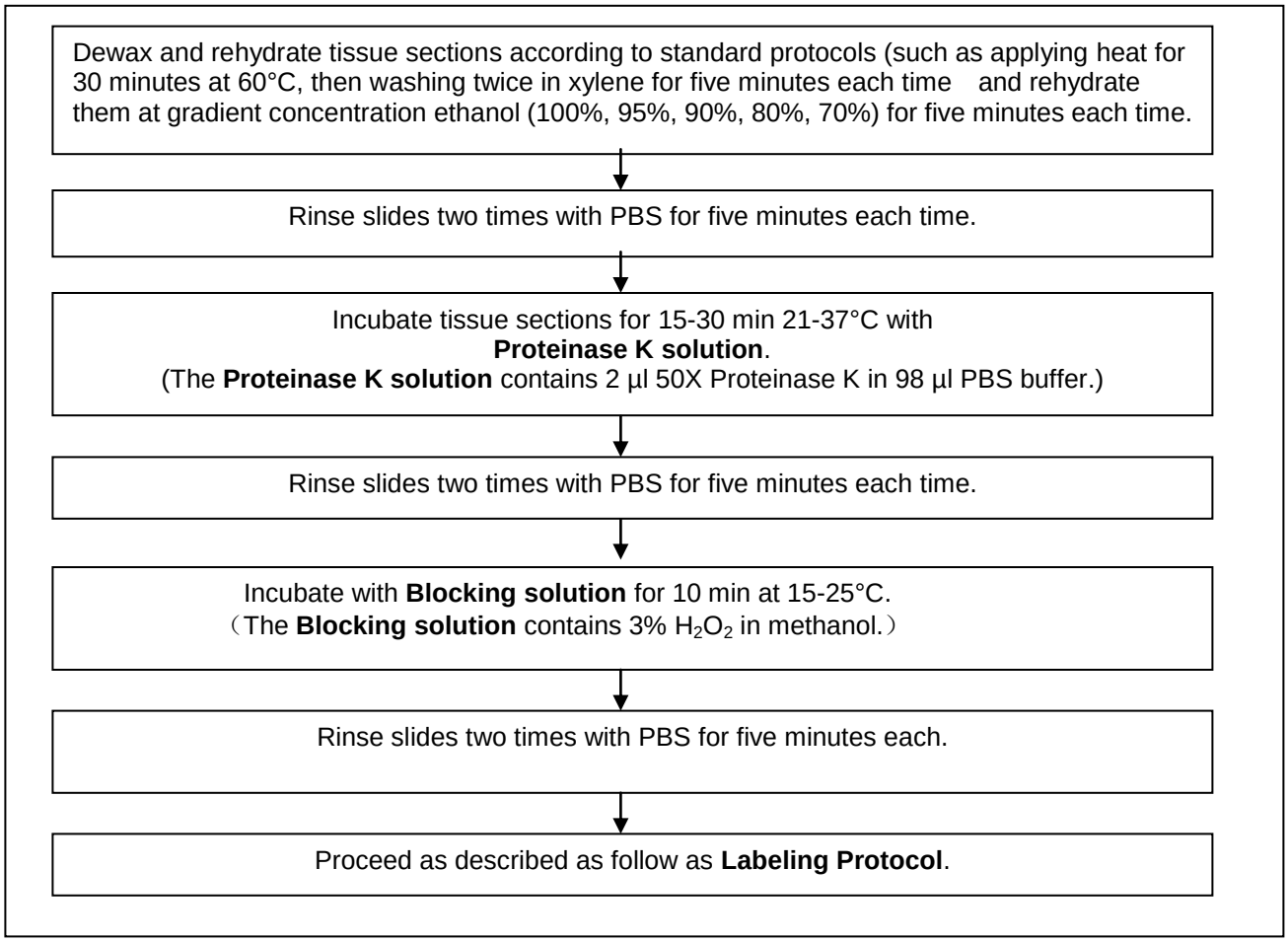
1. Preparing Paraffin-embedded Tissue Sections



* Alternative Treatments

There are other methods of preparing Paraffin-embedded Tissue Sections:

1. Incubate the dewaxed and rehydrated tissue sections with **Permeabilization solution** for 8-10 minutes**.** The **Permeabilization Solution** contains 0.1% Triton X-100 and 0.1% sodium citrate, freshly prepared.

2. Incubate the dewaxed and rehydrated tissue sections with **Pepsin Buffer*** or **Trypsin Buffer***, For 8-10 minutes.

Pepsin Buffer* contains 0.25%-0.5% pepsin in HCl buffer, pH 2.0

Trypsin Buffer* contains 0.25%-0.5% trypsin in 0.01 M HCl buffer.

3. Incubate the dewaxed and rehydrated tissue sections with 200 ml 0.1 M Citrate Buffer (pH 6.0) in a plastic jar. Irradiate with 350 W microwaves for five minutes.

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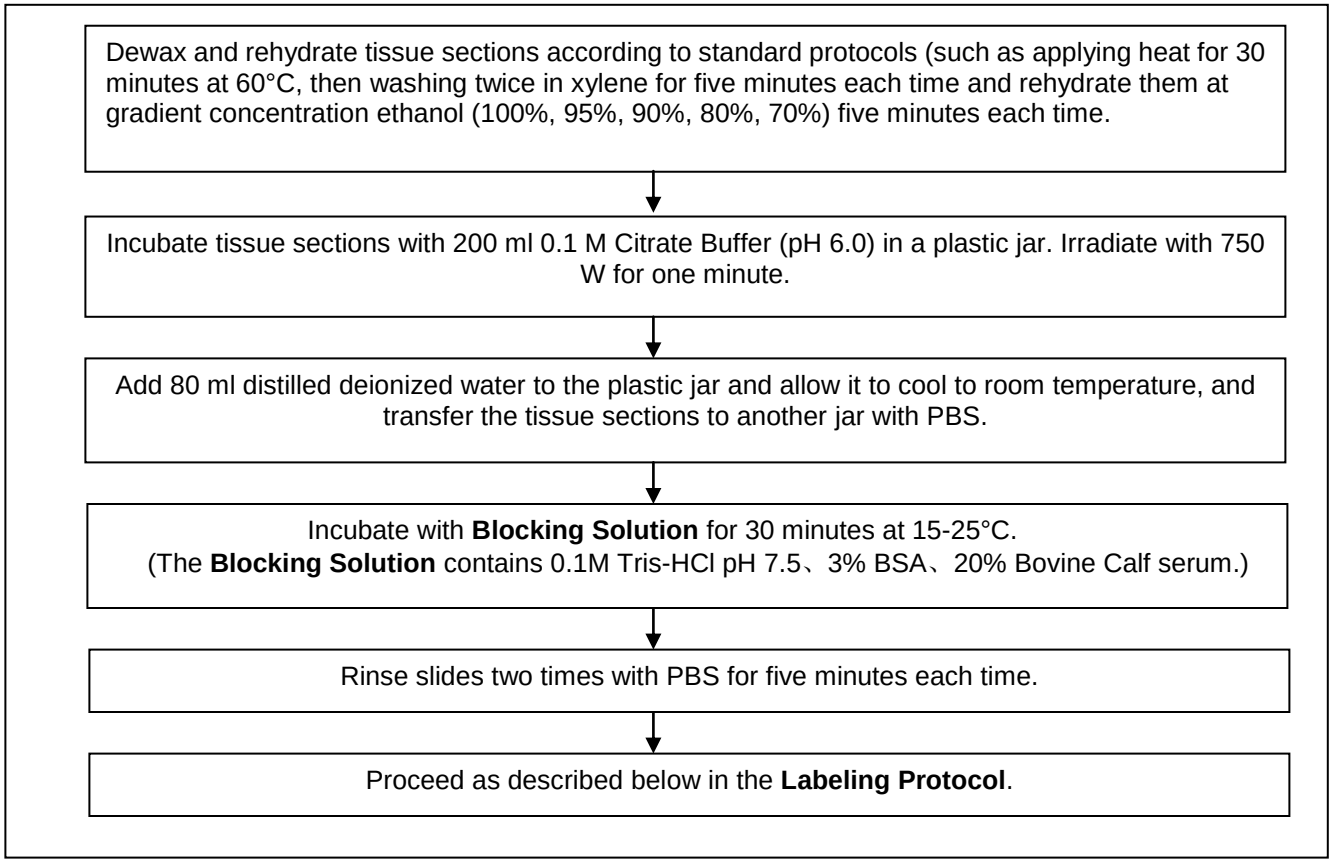
2. Preparing Particular Paraffin-embedded Tissue Sections



Controls:

Negative control: Employ the cells or sections as described the labeling protocol. Label solution but do not add any Terminal Deoxynucleotidyl Transferase (TdT) to the TUNEL Reaction Mixture.

Positive control: Before beginning the labeling procedures, incubate the fixed and permeabilized cells or sections with 100 µl **DNase I Solution** for 10 minutes at 15-25°C to induce DNA strand degradation.

(**DNase I Solution** contains 30000 U/ml-50000 U/ml DNase I (grade I) depending on the sample to be stained in 1X DNase I buffer. One example of 1X DNase I buffer is 10 mM $CaCl_2$, 6 mM $MgCl_2$, and 10 mM NaCl in 40 mM Tris-HCl, pH 7.9)

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Labeling Protocol:

Rinse slides with PBS two times for five minutes each time and then keep the area around the samples dry.

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Add 50 µl **TUNEL Reaction Mixture** to samples. Add a coverslip and incubate for 60 minutes at 37°C under wet conditions, protected from light.
(The **TUNEL Reaction Mixture** contains 45 µl Equilibration Buffer, 1 µl FITC-12-dUTP, and 4 µl TdT, freshly prepared.)
Note: Add 50 µl Label Solution to the negative control. To ensure a homogeneous dispersal of TUNEL Reaction Mixture across the cell monolayer and to avoid loss to evaporation, the samples should be covered with parafilm or a coverslip during incubation.

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Rinse slides with PBS three times for five minutes each time.

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Assay with a fluorescence microscope using excitation wave 450-500 nm and emission wave 515-565 nm (green).

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Keep the area around the samples dry with filter paper.

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Add 50 µl **Anti-fluorescein Antibody Solution** on samples. Incubate slide under wet conditions for 30 minutes at 37°C.
(The **Anti-fluorescein antibody solution** contains 10 µl anti-fluorescein antibody in 40 µl PBS.)
***Note*:** To ensure the homogeneous dispersal of Anti-fluorescein Antibody Solution across the cell monolayer and to avoid loss to evaporation, the samples should be covered with parafilm or a coverslip during incubation.

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Rinse slide three times with **PBS** for two minutes each time.

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Add 50-100 µl **DAB Substrate** and incubate slide for 10 minutes at 15-25°C.
(The **DAB Substrate** contains 5 µl **20X DAB buffer*** and 1 µl 30% H_2O_2 in 94 µl PBS buffer, freshly prepared.)

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Rinse slides three times with PBS. Mount under a glass coverslip (such as with PBS/glycerol) and analyze with light microscope.
(**Alternative:** Samples can be counterstained prior to analysis by light microscope.)

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***20X DAB buffer（10 mg/ml DAB buffer)** contains 10 mg DAB dissolved in 1.0 ml PBS buffer.

VI RELATED PRODUCTS

TUNEL Universal Apoptosis Detection Kit (Biotin labeled POD), Cat. No. L00290

TUNEL Apoptosis Detection Kit for Adherent Cells (Biotin labeled POD), Cat. No. L00296

TUNEL Apoptosis Detection Kit for Paraffin-embedded Tissue Sections (Biotin labeled POD), Cat. No. L00297

TUNEL Apoptosis Detection Kit for Adherent Cells (FITC labeled POD), Cat. No. L00299

TUNEL Apoptosis Detection Kit for Cryopreserved Tissue Sections (FITC labeled POD), Cat. No. L00301

VII. TROUBLESHOOTING

TUNEL Dilution Buffer* contains 150 mM KCl, 1 mM 2-mercaptoethanol, and 50% glycerol in 60 mM KPB , pH 7.2

Problem	Step/Reagent	Possible cause	Solution
High background	Fixation	Formalin fixation leads to a yellowish stain in cells containing melanin precursors.	Use methanol for fixation. However, this may lead to reduced sensitivity.
	TUNEL reaction	The concentration of the labeling mix is too high.	Reduce concentration of labeling mix from 10% to 50%.
	Converter solution	There is endogenous peroxidase activity.	Prior to cell permeabilization, block endogenous peroxidase by incubating for 10 minutes in methanol containing 3% H_2O_2 at 15-25°C.
		Streptavidin-HRP has engaged in non-specific binding.	• Block with anti-mouse serum. • Block with PBS containing 3% BSA for 20 minutes. • Reduce the concentration of Streptavidin-HRP Solution to 50%.
		The DAB incubation time is too long.	Reduce the time of incubation.
	Sample	Mycoplasma contamination	Use a mycoplasma detection kit.
		Highly proliferating cells	Double staining with Annexin-V-Fluos* or a similar substance. **Note**: High background may make measuring with microplate readers impractical.

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Non-specific staining	Fixation	After fixation, nuclease activity is still high.	Block with the buffer containing dUTP and dATP
	TUNEL reaction	The concentration of TdT is too high.	Reduce concentration of TdT from 10% to 50% with **TdT dilution buffer*.**
Low rate of labeling	Fixation	Ethanol and methanol can lead to diminished labeling (chromatins are not cross-linked with proteins during fixation; they are lost during the procedure steps).	Fixate using 4% paraformaldehyde buffer, formalin, or glutaraldehyde.
		Extensive fixation leads to excessive cross-linkage with proteins.	Reduce fixation time or fix by using 2% paraformaldehyde PBS buffer (pH 7.4).
	Paraffin-embedding	Not enough reagent has been used.	•Treat tissue sections after dewaxing with proteinase K (concentration, time, and temperature must be optimized for each type of tissue). •Try microwave irradiation at 370 W (low) for five minutes in 200 ml 0.1 M Citrate Buffer pH 6.0 (These must be optimized for each type of tissue).
	Permeabilization	The permeabilization step is too short and the reagents can't reach their target molecules.	• Increase the incubation time. • Incubate at a higher temperature (such as 15-25°C). • Optimize the concentration and action time of proteinase K. (e.g. 400ug/ml for 5 minutes) • Incubate with 0.1 M sodium citrate at 70°C for 30 minutes.
No signal in positive control	DNase treatment	The concentration of DNase I buffer is too low.	• Incubate with 30000 U/ml DNase I Solution* or higher for 30 min at 37°C, and then rinse by PBS.
Weak signals	Counterstaining	The dye is not suitable.	Counterstain with 3-5% methyl green in 0.1 M veronal acetate, pH 4.0 or Hematoxylin.
Tissue Sections fall off.	Permeabilization	Tissue Sections are degested by Proteinase K.	Reduce the incubation time of Proteinase K.

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VIII. ORDERING INFORMATION

TUNEL Apoptosis Detection Kit for Paraffin-embedded Tissue Sections (FITC labeled POD), Cat. No. L00300

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