

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

Technical Manual No. 0267

Version 03112011

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I. DESCRIPTION

TUNEL Universal Apoptosis Detection Kit (Biotin-labeled POD) (Cat. No. L00290) is one of GenScript's newly introduced products. It is used for assaying adherent Cells, paraffin-embedded tissue sections and cryopreserved tissue sections. The Kit could detect the fragmented DNA in the nucleus in the period of apoptosis. In this modified TUNEL assay kit, biotinylated nucleotide is labeled at the DNA 3'-OH ends using the natural or recombinant Terminal Deoxynucleotidyl Transferase (TdT or rTdT). And then horseradish peroxidase-labeled streptavidin (Streptavidin-HRP) is bound to these biotinylated 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 FEACTURES

- **Simplified Procedure:** The kit contains Ready-to-use reagents, especially Proteinase K, DAB and DNase I
- **Enhanced Sensitivity:** The kit could assay the cells in early period of apoptosis.
- **Enhanced Specificity:** The kit could stain apoptosis cells.
- **Streamlined Process:** The whole process is about three hours.
- **Increased Observation:** The result can be observed by light microscope.
- **High Veracity:** The kit contains positive control reagent.

III. KIT CONTENTS

TUNEL Universal Apoptosis Detection Kit (L00290) employs biotinylated nucleotide (Biotin-11-dUTP), horseradish peroxidase-labeled streptavidin, TdT, Proteinase K and DAB.

Components	Cat. No. L00290 20 Assays	Cat. No. L00290 50 Assays	Cat. No. L00290 100 Assays	Storage Conditions
Equilibration Buffer	1.0 ml	2.5 ml	5.0 ml	-20°C
Biotin-11-dUTP	20 µl	50 µl	100 µl	-20°C
TdT	80 µl	200 µl	400 µl	-20°C
50X Proteinase K (1mg/ml)	40 µl	100 µl	200 µl	-20°C
Streptavidin-HRP	10 µl	25 µl	50 µl	4°C, store away from light
DAB	2 mg	5 mg	10 mg	-20°C
DNase I (50 U/µl)	1 ml	1 ml	1 ml	-20°C
1×DNase I buffer	1 ml	1 ml	1 ml	4°C

IV. STORAGE

Store Streptavidin-HRP at 4°C, and do not expose to light. Store DNase I buffer at 4°C. Store the rest of the kit at -20°C. It will remain stable for one year.

V. PROCEDURE

Before use, order or prepare the following:

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

Blocking Solution: 3% H₂O₂ in methanol. e.g. 1ml 30% H₂O₂ + 9ml methanol.

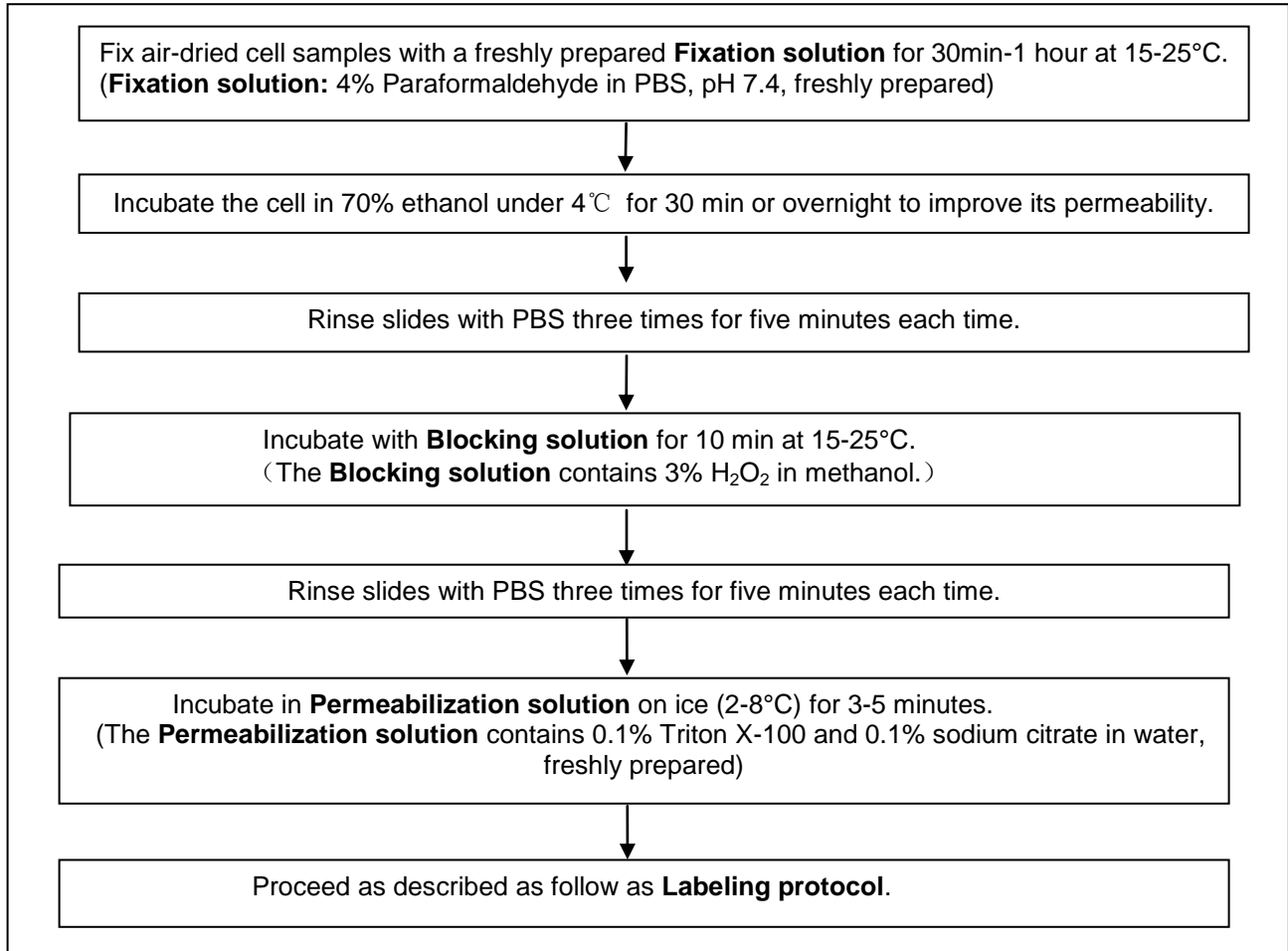
Permeabilization Solution: 0.1% Triton X-100 and 0.1% sodium citrate in water, freshly prepared.

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 20X DAB buffer (10 mg/ml DBA buffer) before use.

1. Preparation of Sample Material

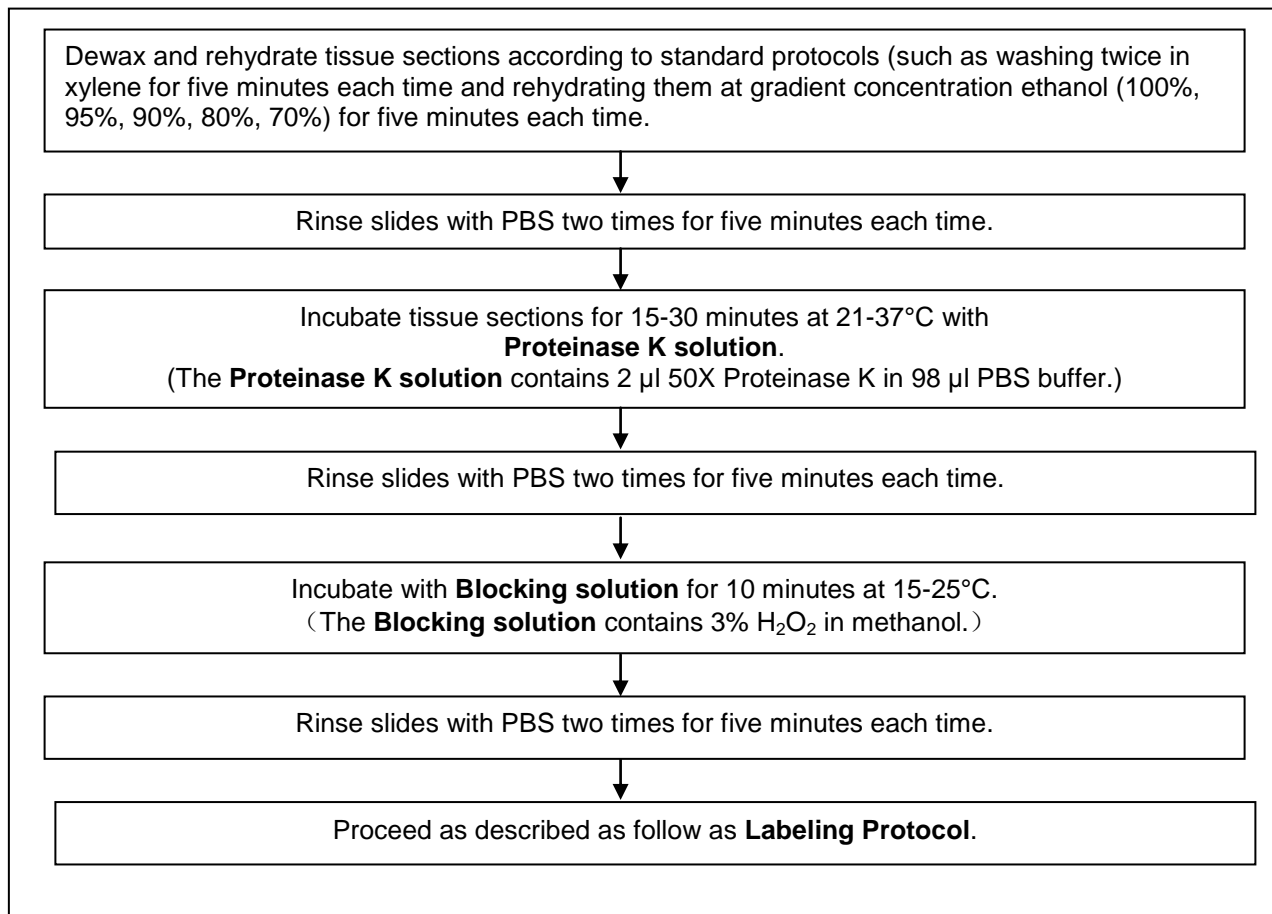
1.1 Adherent cells, cell smears and cytospin preparations



1.2 Paraffin-embedded Tissue Sections

Specification: For paraffin-embedded sections, the suitable thickness is about 5 µm, but no more than 10 µm. If the paraffin section is thicker than 10 µm, the kit may not work well.

1.2.1 Preparing conventional 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.

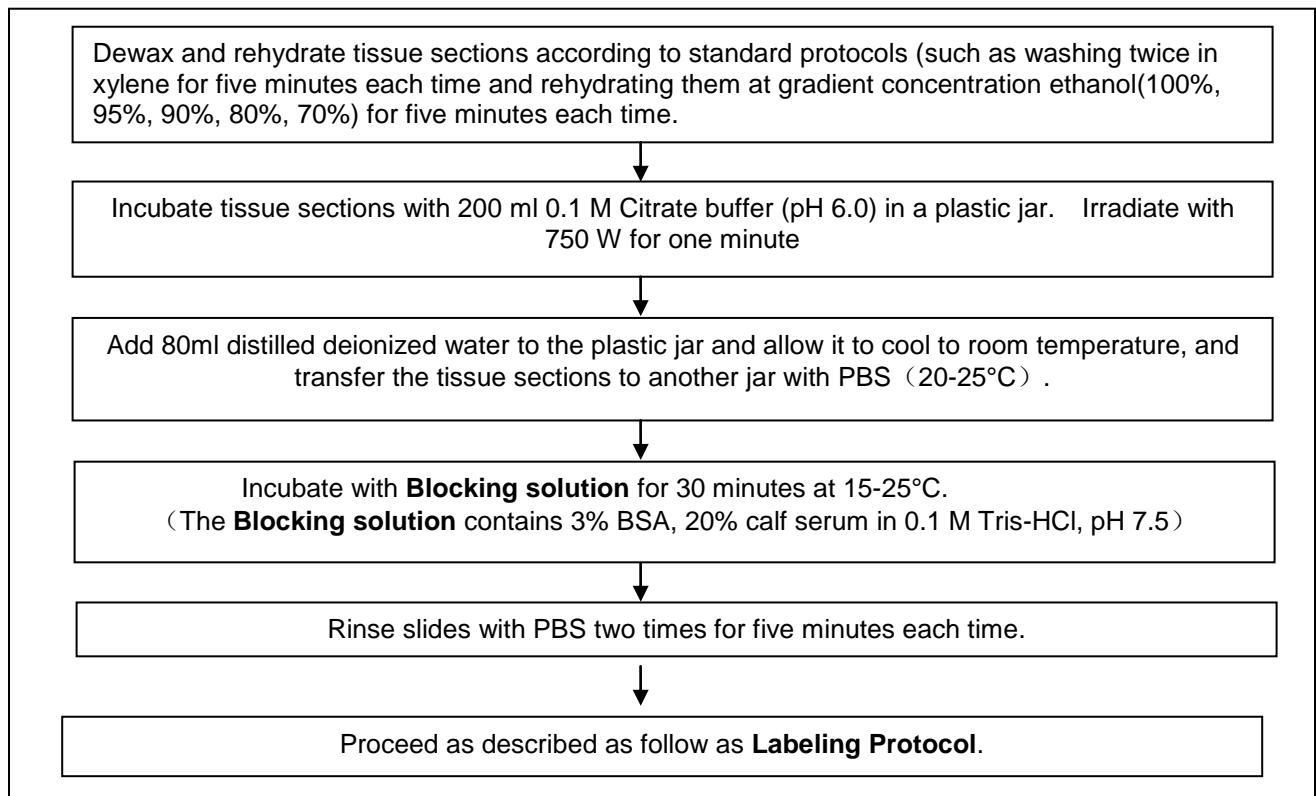
2. Incubate the dewaxed and rehydrated tissue sections with **Pepsin Buffer*** or **Trypsin Buffer*** for 8-10 minutes.

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.

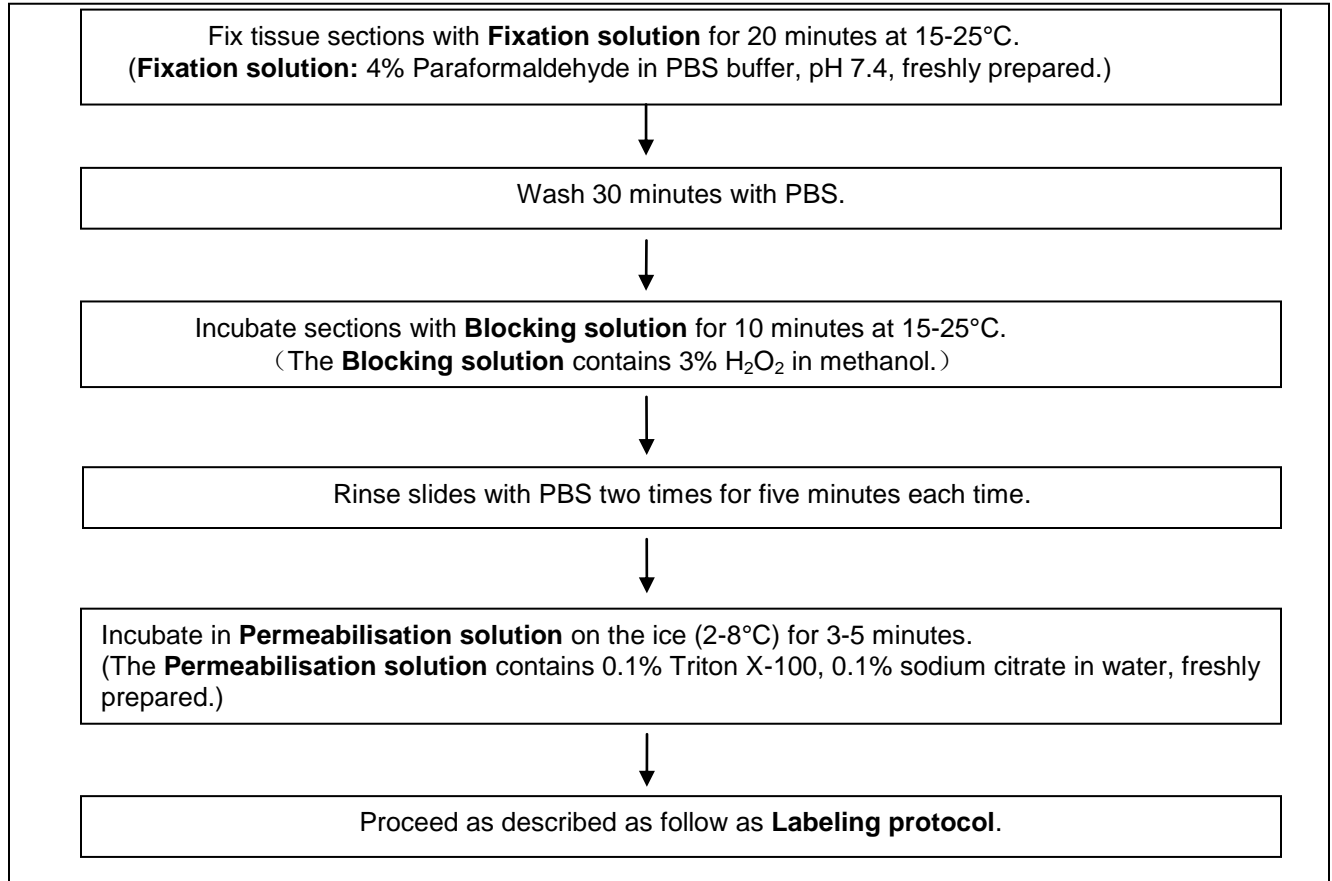
3. Incubate the dewaxed and rehydrated tissue sections with 200 ml 0.1 M Citrate Buffer (pH6.0) in a plastic jar. Irradiate with 350 W microwaves for five minutes.

1.2.2 Preparing particular Paraffin-embedded Tissue Sections



1.3 Cryopreserved Tissue sections

Specification: The kit is suitable for 10-30 μm cryopreserved section. If the cryopreserved section is thicker than 30 μm , the kit may not work well.



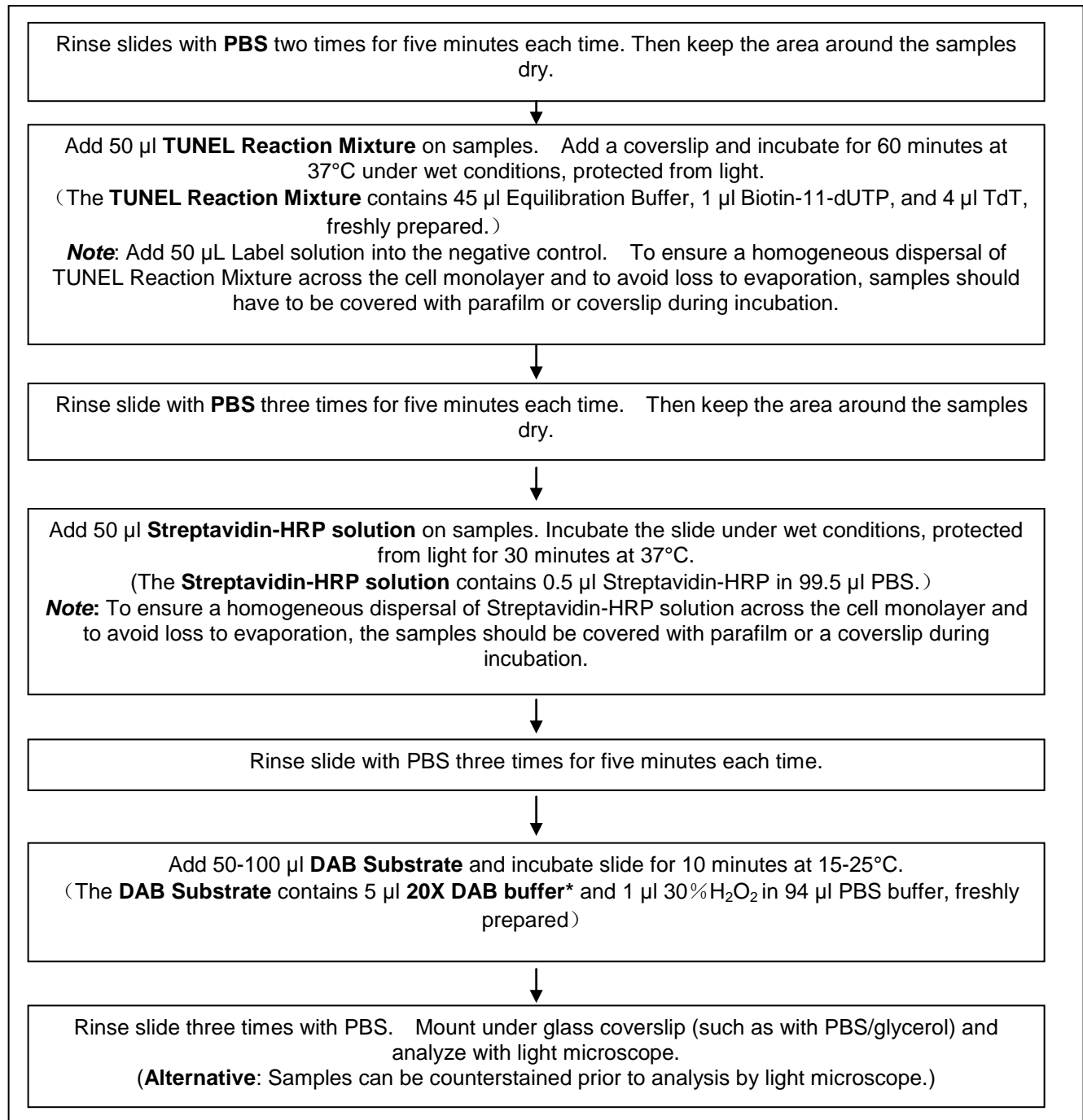
Controls:

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

Positive control: Before beginning the labeling procedure, incubate the fixed and permeabilized cells or sections with 100 μl **DNase I Solution** for 10 -30 minutes at 21-37°C to induce DNA strand degradation.

DNase I Solution contains 10000 U/ml-50000 U/ml DNase I (grade I) depending on the sample to be stained in 1 \times DNase I buffer (the concentration of DNase I is 10000 U/ml– 20000 U/ml for cell sample, 20000U/ml – 30000U/ml for cryopreserved section, and 30000U – 50000U/ml for paraffin-embedded sections). One example of 1 \times DNase I buffer is 10 mM CaCl_2 , 6 mM MgCl_2 , and 10 mM NaCl in 40 mM Tris-HCl, pH 7.9

2. Labeling protocol



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

VI. RELATED PRODUCTS

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 Paraffin-embedded Tissue Sections (FITC labeled POD), Cat. No. L00300

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

VII. TROUBLESHOOTING

Problem	Step/Reagent of Procedure	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 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 ₂ O ₂ at 15-25°C.
		Streptavidin-HRP has engaged in non-specific binding.	<ul style="list-style-type: none"> Block with anti-mouse serum. Block with PBS containing 3% BSA for 20 min. Reduce 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.

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 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).	Fix using 4% paraformaldehyde buffer or formalin or glutaraldehyde.
		Extensive fixation leads to excessive cross-linked with proteins	Reduce fixation time, or fix by using 2% paraformaldehyde PBS buffer (pH 7.4).
	Permeabilization	Permeabilization is too short so that reagents can't reach their target molecules.	<ul style="list-style-type: none"> • Increase incubation time. • Incubate at 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.
	Paraffin-embedding	Not enough reagent has been used.	<ul style="list-style-type: none"> • 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 5 minutes in 200 ml 0.1 M Citrate Buffer, pH 6.0 (These must be optimized for each type of tissue).
No signal in positive control	DNase treatment	The concentration of DNase I buffer is too low.	<ul style="list-style-type: none"> • Incubate with 30000 U/ml DNase I Solution* or higher for 30 minutes at 37°C, and then rinse with 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	The tissue sections have been digested by proteinase K.	Reduce the time in which of proteinase K is permitted to act.

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

VIII. ORDERING INFORMATION

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

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