TUNEL Universal Apoptosis Detection Kit (FITC-labeled) **Cat. No. L00427**

Technical Manual No. TM0623 **Version 03102011**

I. DESCRIPTION

TUNEL Universal Apoptosis Detection Kit (FITC-labeled) (**Cat. No. L00427**) is used for the fast detection of fragmented DNA in the nucleus during apoptosis. In this modified TUNEL assay kit, fluorescein-labeled nucleotides binds with the DNA 3´-OH ends using natural or recombinant terminal deoxynucleotidyl transferase (TdT or rTdT). The fluorescence could be observed by fluorescence microscope.

II. KEY FECTURES

- **Simplified Procedure:** The kit contains ready-to-use reagents, only need one step of staining procedure.
- **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 1-2 hours.
- **Increased Convenience:** The kit can be used for adherent Cells, paraffin-embedded tissue sections and cryopreserved tissue sections.
- **High Veracity:** The kit contains positive control reagent.

III. KIT CONTENTS

The TUNEL Apoptosis Detection Kit is available. L00427 is for detection using fluorescein Labeled nucleotides (FITC-12-dUTP).

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Components	Cat. No.L00427 20 Assays	Cat. No.L00427 50 Assays	Cat. No. L00427 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
50×Proteinase K (1 mg/ml)	40 µl	100 µl	200 µl	-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 the kit at -20°C. It will remain stable for one year.

V. PROTOCOL

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. e.g 1ml 30% H_2O_2 + 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.

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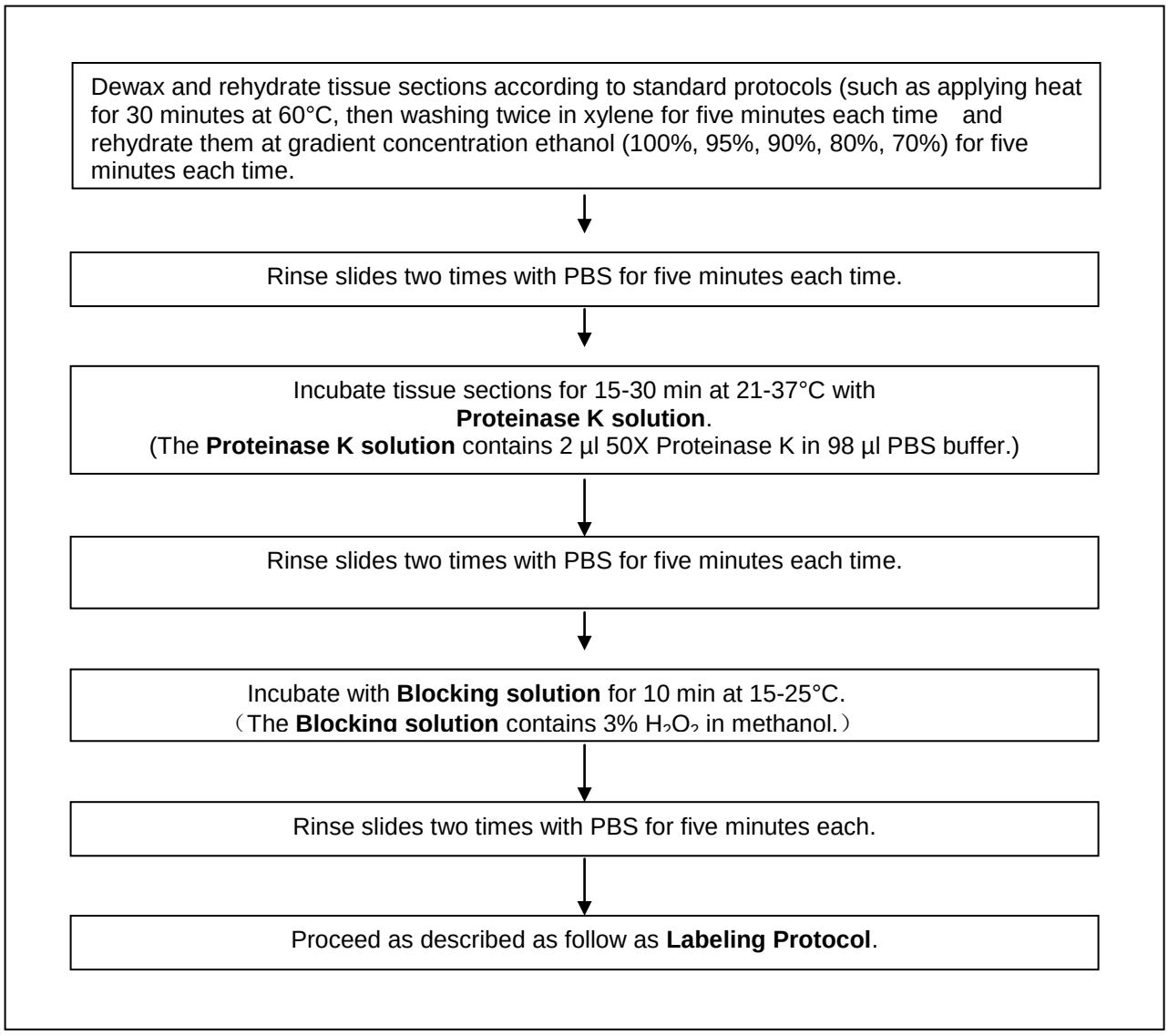
1. Preparing Paraffin-embedded Tissue Sections:



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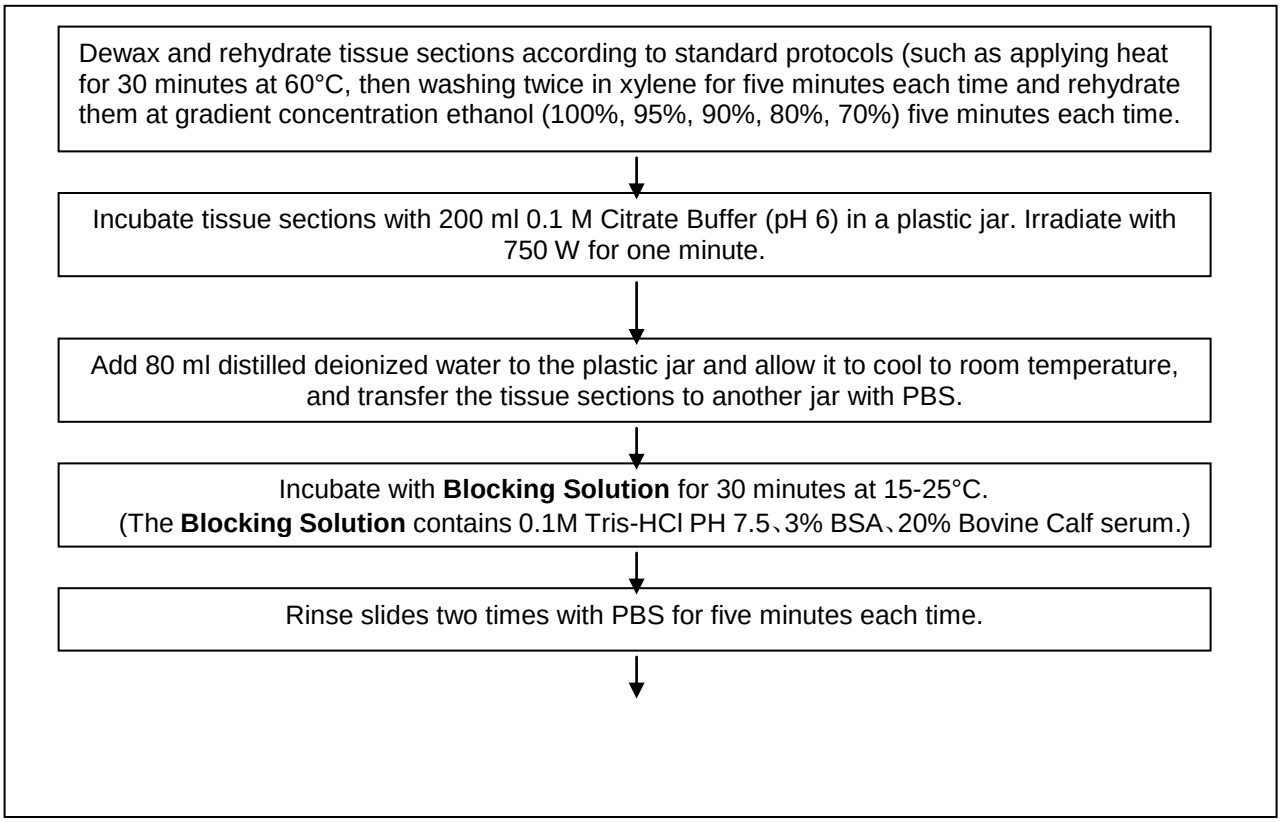
2. Preparing Particular Paraffin-embedded Tissue Sections



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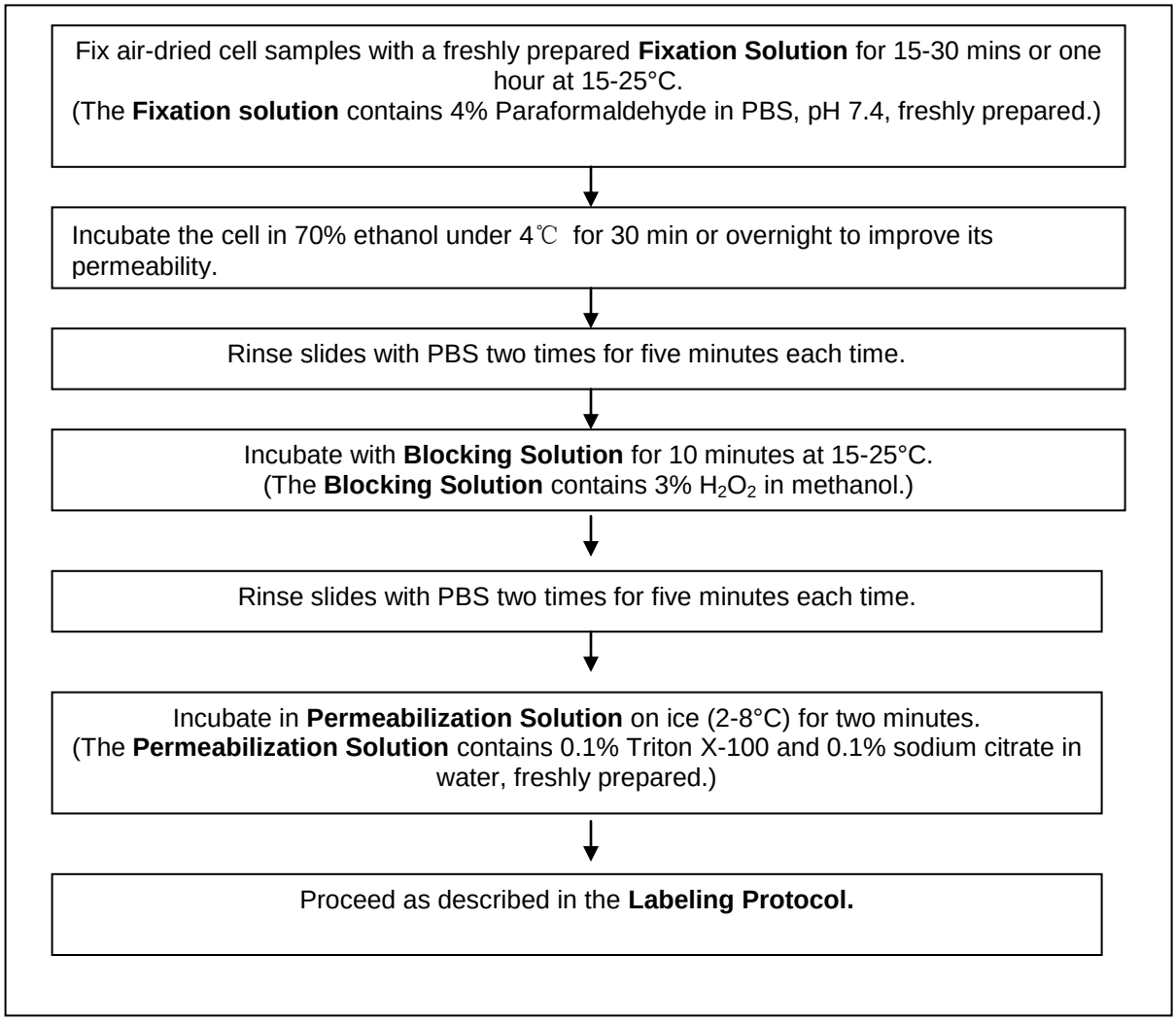
3. Adherent Cells, Cell Smears, and Cytospin Preparations



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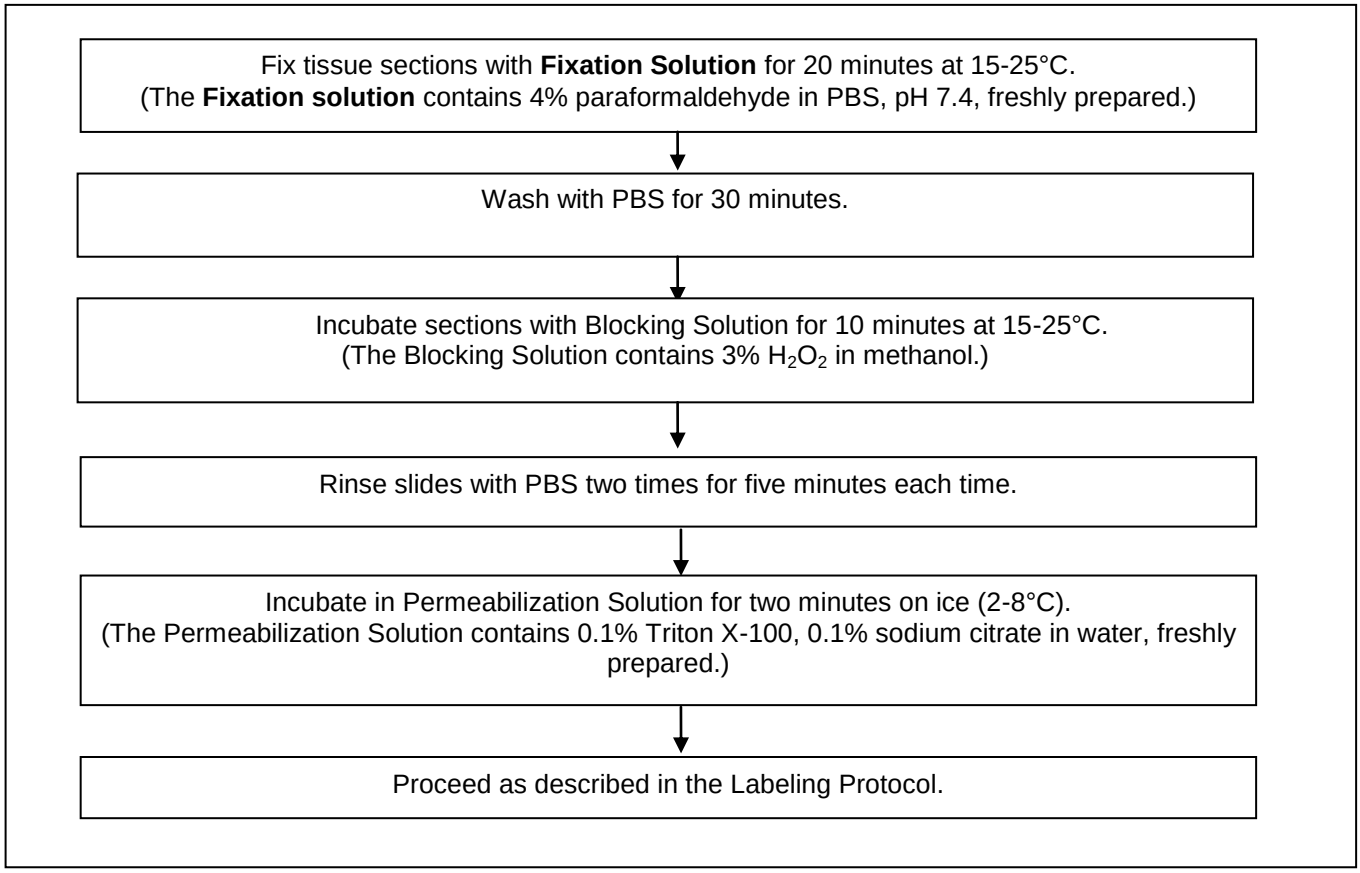
4. Cryopreserved Tissue Sections



Controls:

Negative control: Employ the cells or sections as described the labeling protocol. Label solution but do not add any Terminal Deoxynucleotidyl Transferase (TdT) 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× DNase I buffer (the concentration of DNase I is 10000 – 20000 U/ml for cell sample, 20000U/ml – 30000U/ml for cryopreserved section,and 30000U – 50000U/ml for paraffin-embeded sections). One example of 1× 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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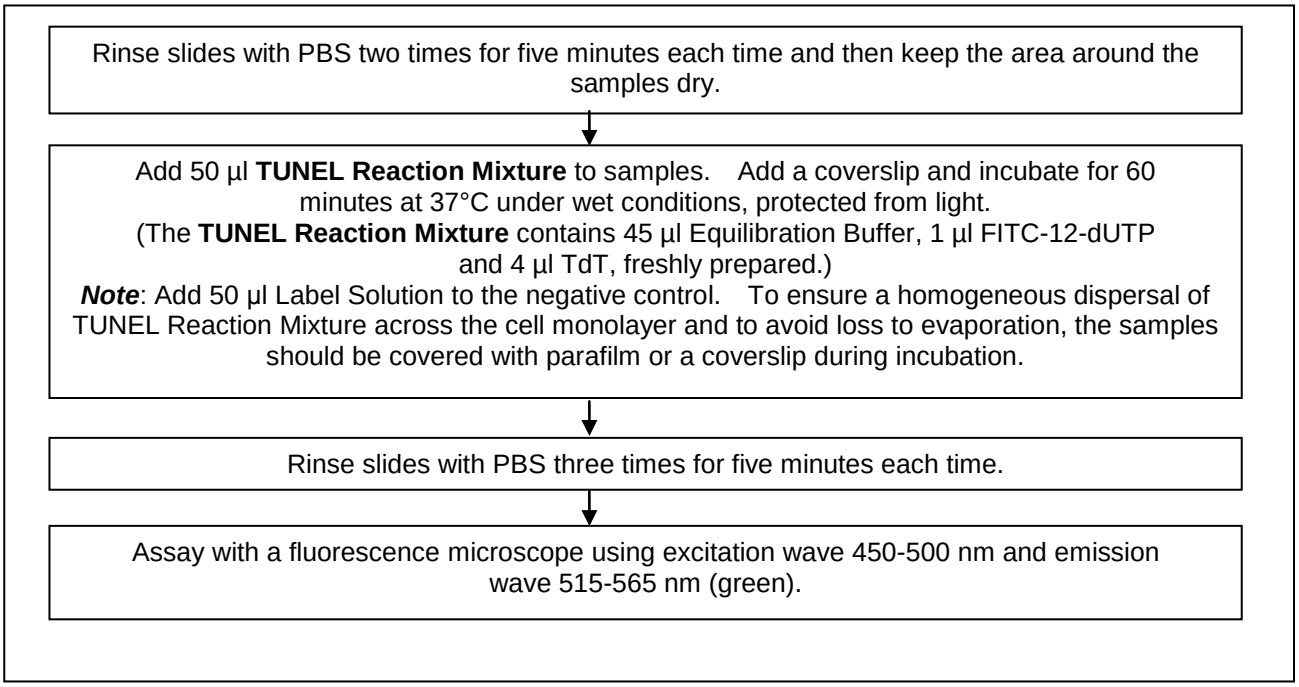
Labeling Protocol



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

TUNEL Universal Apoptosis Detection Kit (For cell tissue section, TRITC-labeled), Cat. No. L00426

TUNEL Two-Color Apoptosis Detection Kit (FITC-labeled, for flow cytometry), Cat. No. L00428

TUNEL Two-Color Apoptosis Detection Kit (TRITC-labeled, for flow cytometry), Cat. No. L00429

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VII. TROUBLESHOOTING

TdT 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.
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).

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	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. • Incubate with 0.1 M sodium citrate at 70°C for 30 minutes.
No signal on positive control	DNase treatment	The concentration of DNase I Buffer is too low.	• Incubate with 30000U/ml DNase I Solution* or higher for 30 min at 37°C, and then rinse with PBS.
Weak signals	Counterstaining	The dye is not suitable.	Counterstain with 5% methyl green in 0.1 M veronal acetate, pH 4.0 or Hematoxilin.

VIII. ORDERING INFORMATION

TUNEL Universal Apoptosis Detection Kit (For cell tissue section, FITC-labeled), Cat. No. L00427

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