

**TUNEL Apoptosis Detection Kit  
 (For Cryopreserved Tissue Sections, FITC-labeled POD)**
**Cat. No. L00301**
**Technical Manual No. 0269**
**Version 01132011**

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**I. DESCRIPTION**

**TUNEL Apoptosis Detection Kit for Cryopreserved Tissue Sections (FITC-labeled POD) (Cat. No. L00301)** is used for the 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. 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 FECTURES**

- **Simplified Procedure:** The kit contains ready-to-use reagents, including DAB.
- **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** is available. L00301 is for detection using fluorescein Labeled nucleotides (FITC-12-dUTP), HRP-labeled anti-FITC antibody, TdT and DAB.

Components	Cat. No.L00301 20 Assays	Cat. No.L00301 50 Assays	Cat. No. L00301 100 Assays	Storage Conditions
Equilibration Buffer	1ml	2.5ml	5.0 ml	-20°C
FITC-12-dUTP	20ul	50ul	100 µl	-20°C
TdT	80ul	200ul	400 µl	-20°C
HRP-labeled Anti-FITC Antibody	200ul	500ul	1000 µl	-20°C
DAB	2mg	5mg	10 mg	-20°C
DNase I (50 U/µl)	0.2ml	0.5ml	1ml	-20°C
1 × DNase I buffer	0.2ml	0.5ml	1ml	4°C

#### IV. STORAGE

Store the kit at -20°C. It will remain stable for one year.

#### V. PROTOCOL

**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.

**Before use, order or prepare the following:**

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

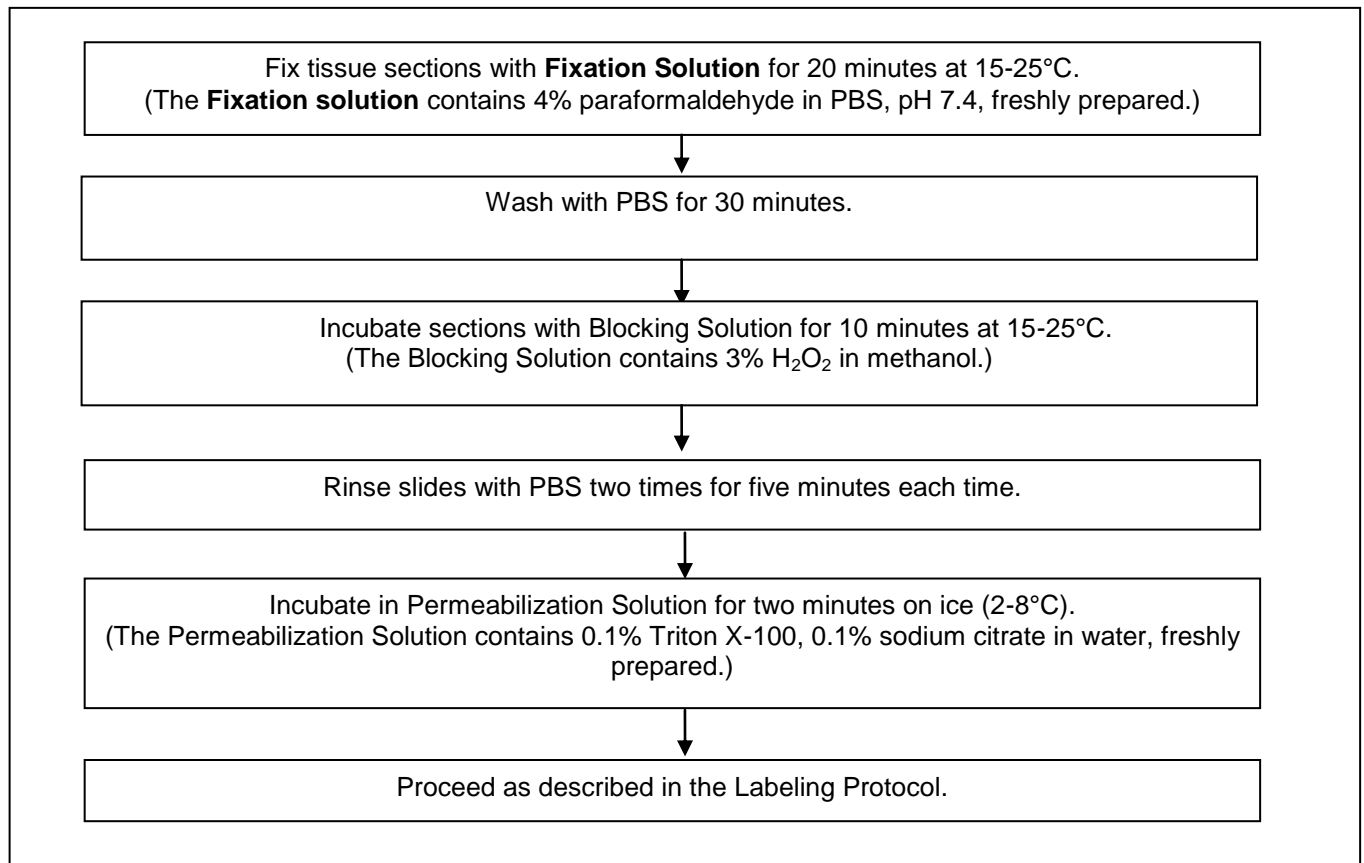
Blocking Solution: 3% H<sub>2</sub>O<sub>2</sub> in methanol. e.g. 1ml 30% H<sub>2</sub>O<sub>2</sub> + 9ml methanol.

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

## Cryopreserved Tissue Sections



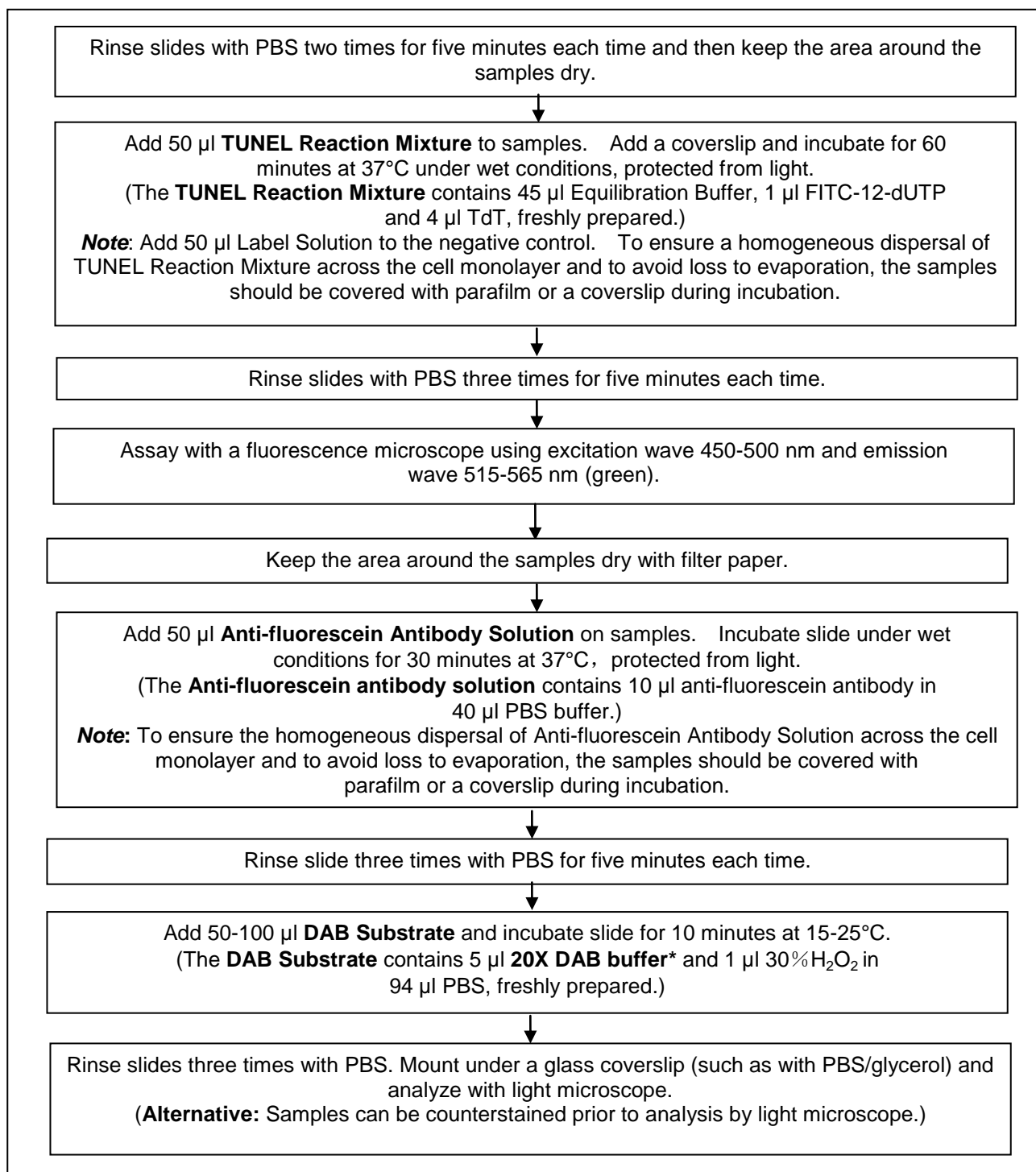
### Controls:

**Negative control:** Employ the cells or sections as described the labeling protocol. Label solution but do not add any Terminal Deoxynucleotidyl Transferase (TdT) 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 20000 U/ml-30000 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<sub>2</sub>, 6 mM MgCl<sub>2</sub>, and 10 mM NaCl in 40 mM Tris-HCl, pH 7.9)

## Labeling Protocol



\***20X DAB buffer (10 mg/ml DAB buffer)** contains 10 mg DAB dissolved in 1 ml PBS.

## 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

## 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 <sub>2</sub> O <sub>2</sub> at 15-25°C.
			Streptavidin-HRP has engaged in non-specific binding.	<ul style="list-style-type: none"> <li>Block with anti-mouse serum.</li> <li>Block with PBS containing 3% BSA for 20 minutes.</li> <li>Reduce the concentration of Streptavidin-HRP Solution to 50%.</li> </ul>
			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. <b>Note:</b> 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 <b>TdT dilution buffer*</b> .	

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).
	Permeabilization	The permeabilization step is too short and the reagents can't reach their target molecules.	<ul style="list-style-type: none"> <li>• Increase the incubation time.</li> <li>• Incubate at a higher temperature (such as 15-25°C).</li> <li>• Optimize the concentration and action time of proteinase K. (e.g. 400ug/ml for 5 minutes)</li> <li>• Incubate with 0.1 M sodium citrate at 70°C for 30 minutes.</li> </ul>
No signal on positive control	DNase treatment	The concentration of DNase I Buffer is too low.	<ul style="list-style-type: none"> <li>• Incubate with 30000U/ml DNase I Solution* or higher for 30 min at 37°C, and then rinse with PBS.</li> </ul>
Weak signals	Counterstaining	The dye is not suitable.	Counterstain with 5% methyl green in 0.1 M veronal acetate, pH 4.0 or Hematoxylin.

## VIII. ORDERING INFORMATION

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

GenScript USA Inc  
 860 Centennial Ave., Piscataway, NJ 08854  
 Tel: 1-877-436-7472  
 Fax: 1-732-210-0262  
 E-mail: [product@genscript.com](mailto:product@genscript.com)  
 Web: [www.genscript.com](http://www.genscript.com)

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