

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

Technical Manual No.0262

Version 01132011

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I. DESCRIPTION

The **TUNEL Apoptosis Detection Kit for Adherent Cells (Biotin-labeled POD) (Cat. No.: L00296)** is one of GenScript's newly introduced products. It is used for detecting 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 FEATURES

- **Simplified Procedure:** The kit contains ready-to-use reagents, including 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 light microscope.
- **High Veracity:** The kit contains positive control reagent.

III. KIT CONTENTS

The **TUNEL Apoptosis Detection Kit (L00296)** employs biotinylated nucleotide (biotin-11-dUTP), horseradish peroxidase-labeled streptavidin, TdT, and DAB.

Components	Cat. No. L00296 20 Assays	Cat. No. L00296 50 Assays	Cat. No. L00296 100 Assays	Storage Conditions
Equilibration Buffer	1.0ml	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
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)	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 streptavidin-HRP at 4°C, and do not expose it to light. Store the rest of the kit at -20°C. It will remain stable for one year.

V. TUNEL APOPTOSIS DETECTION KIT PROCEDURE:

Before use, order or prepare the following:

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

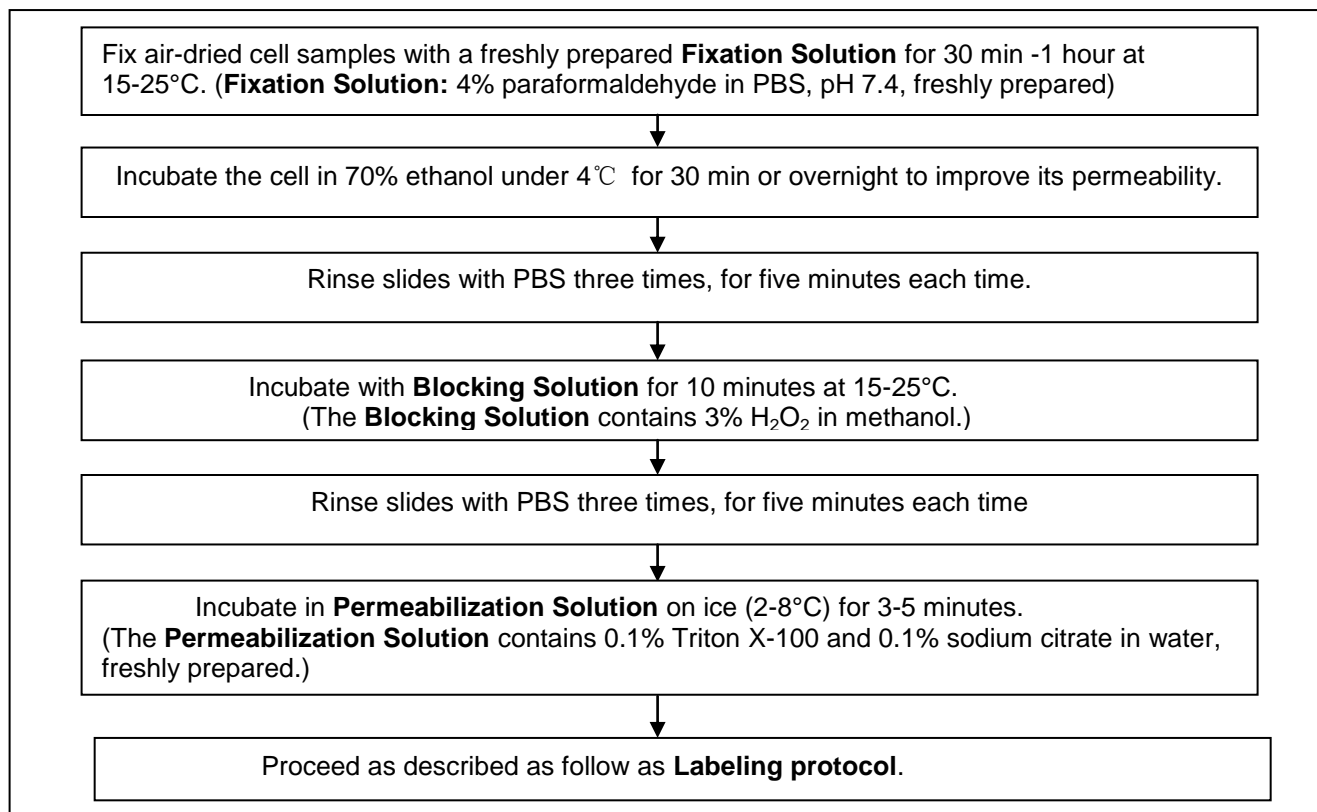
Blocking Solution: 3% H₂O₂ in methanol. e.g. 1ml 30% H₂O₂ + 9ml methanol.

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

Adherent Cells, Cell Smears, and Cytospin Preparations



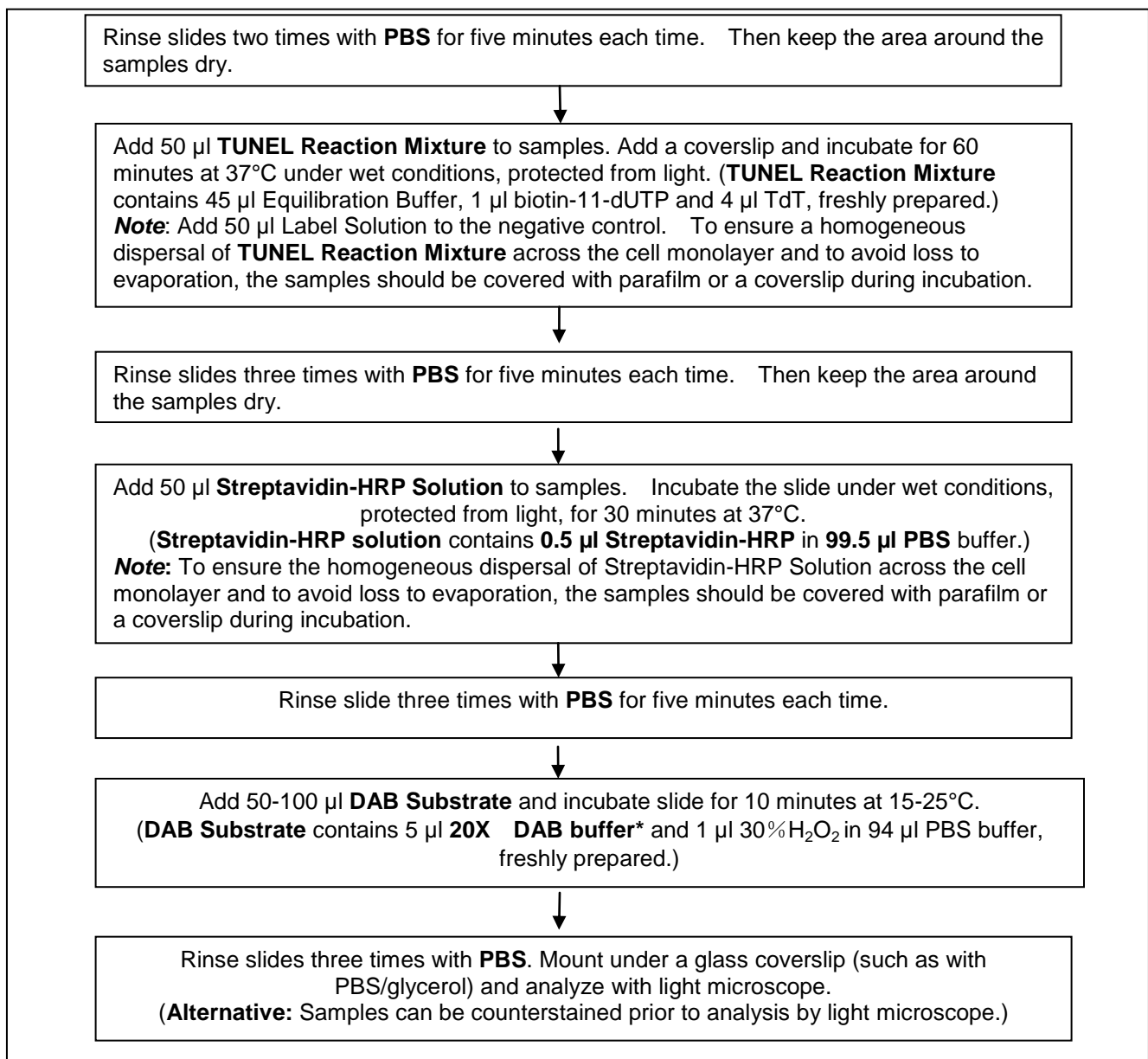
Labeling Protocol:

Controls:

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

Positive control: Before beginning the labeling procedures, incubate the fixed and permeabilized cells with 100 μ l DNase I Solution for 10-30 minutes at 15-37°C to induce DNA strand degradation.

(**DNase I Solution** contains 10000 U/ml-20000 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 CaCl₂, 6 mM MgCl₂, and 10 mM NaCl in 40 mM Tris-HCl, pH 7.9



*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 for Paraffin-embedded Tissue Sections (Biotin-labeled POD), Cat. No. L00290

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	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 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 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.	

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"> • 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.	<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.
Non-specific staining	Fixation	After fixation, nuclease activity is still high.	Block with 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.

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

VIII. ORDERING INFORMATION

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

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