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FD ApopTM Kit

**A complete labeling system for the microscopic detection of cells
undergoing apoptosis based on the principle of
in situ DNA nick-end labeling technique**

User Manual

(PK101, Version 2002-05)

FOR *IN VITRO* RESEARCH USE ONLY
not for diagnostic or other uses

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I. DESCRIPTION

FD Apop™ Kit is designed for the microscopic detection of cells undergoing apoptosis based on the principle of *in situ* DNA nick-end labeling technique (TUNEL)¹. The assay uses terminal deoxynucleotidyl transferase to catalyze incorporation of biotinylated deoxyuridines onto the free 3'-hydroxyl termini of DNA breaks, which are considered as one of the most characteristic features of apoptosis^{2, 3}. The integrated biotins are amplified and visualized with the avidin-biotin-complex method⁴, enabling light microscopic identification.

The reagents and procedure of FD Apop™ kit have been optimized to achieve a high degree of both specificity and sensitivity for detecting apoptotic cells with the lowest background. The kit can be used with frozen or paraffin-embedded sections, as well as cultured cells. The procedure of the kit takes approximately 4 hours.

II. KIT CONTENTS

Part 1 (Store at -20°C):	Digestive Enzyme	2 ml x 4
	Reaction Solution A	2 ml x 2
	Reaction Solution B	60 µl
	Reaction Solution C	40 µl
	Chromogen Solution	20 ml
Part 2 (Store at 0-4°C):	Equilibration Buffer	20 ml
	Detection Reagent	6 ml
	10x Phosphate-Buffered Saline	250 ml x 2

III. MATERIALS REQUIRED BUT NOT INCLUDED

1. Double distilled water
2. Microcentrifuge tubes
3. Adjustable micropipettor
4. Humidified chamber
5. Incubator or waterbath (30°C)
6. Histological supplies and equipment, including microscope slides, coverslips, staining jars, cover glass forceps, xylene or xylene substitutes, mounting medium and a light microscope

IV. SAFETY AND HANDLING PRECAUTIONS

1. FD Apop™ kit is made for research use only, not for diagnostic or other uses.
2. The reagents in the kit may be harmful or fatal if ingested. Do not pipette by mouth. Avoid contact with skin and eyes. In case of contact, wash immediately with copious amounts of water and call a physician.
3. Wear disposable gloves while handling kit reagents. Wash hands thoroughly after performing the experiment.

V. TISSUE PREPARATION (for unfixed frozen tissue)

FD Apop™ kit has proven to produce best results in tissue sections prepared according to the following procedures. However, it may be used in tissue specimens prepared differently (please contact FD NeuroTechnologies for more information before use).

1. Unfixed frozen tissue* may be cut on a cryostat at 20 µm and directly mounted on poly-lysine-coated or proteinase-resistant (cf. Cat. #PO102) microscope slides.

***Perfusion of animals and fixation of tissue are not necessary. However, to prevent tissue from ice crystal damage and to preserve the best cell morphology, tissue must be rapidly frozen upon removal, e.g. by immersion of tissue in -70°C isopentane precooled with dry ice. Please contact us for a detailed procedure.**

2. Sections should be dried at room temperature for at least 30 minutes and be stored in slide boxes containing desiccant at -80°C (or -20°C if -80°C is not available) before processing with FD Apop™ kit.

Tip: A small fan may be used to dry sections faster. **DO NOT USE HOT AIR OR HOT PLATE!**

VI. SECTION PRETREATMENT

Note:

1. Labeling procedure (cf. page 4) should be continued once the section pretreatment commences.
2. The following procedure takes approximately 1 hour and should be carried out at room temperature.

For unfixed frozen sections:

1. Place sections in 0.1 M phosphate buffer (pH 7.4) containing 4% paraformaldehyde for 30 minutes (slides stored at -80°C or -20°C should be directly transferred into the fixative solution).
2. Wash sections in 0.01 M phosphate-buffered saline (PBS, pH 7.4) 2 times, 10 minutes each (10x PBS provided, dilute with distilled water before use).

Note:

For best results, sections to be labeled should be encircled with a PAP pen after the first wash.

3. Complete labeling procedure (cf. page 4).

For paraffin sections:

1. Deparaffinize sections in xylene or xylene substitutes, 2 changes, 5 minutes each.
2. Place sections in 100% ethanol, 2 changes, 3 minutes each.
3. Place sections in 95% ethanol for 3 minutes.

4. Place sections in 75% ethanol for 3 minutes.
5. Rinse in distilled water, 3 changes, 3 minutes each
6. Complete labeling procedure (cf. below).

Note:

For the best results, sections to be labeled should be encircled with a PAP pen after the first rinse.

VII. LABELING PROCEDURE

Note:

- Do not let sections dry out during or between any steps.
 - Reagents from Part 1 should be kept on ice during usage.
 - The following procedure takes approximately 3 hours and should be carried out at room temperature unless specifically indicated.
1. Wash sections in 0.01 M PBS for 10 minutes (10x PBS provided, dilute with distilled water before use).
 2. Cover the entire section with **Digestive Enzyme** (approximately 50 μ l for each cm^2 of section to be labeled) and incubate at 30°C for 10 minutes (frozen sections) or 15 minutes (paraffin sections).

Note:

The 10- or 15-minute digestion time is satisfactory in most cases. However, variations in tissue processing may require a shorter or longer time of incubation to obtain the best result. **Very important!**

3. Wash sections in double distilled water 3 times, 3 minutes each.
4. Cover the entire section with **Equilibration Buffer** (approximately 100 μ l for each cm^2 of section to be labeled) and incubate for 5-20 minutes (in the meantime, prepare **Reaction Solution Mixture** for the next step, see below).
5. Cover the entire section with **Reaction Solution Mixture*** (approximately 20 μ l for each cm^2 of section to be labeled) and incubate in a humidified chamber at 30°C for 50 minutes.

***Reaction Solution Mixture:** mix **Reaction Solution A, B and C** in the proportion of 100:3:2 (prepare the mixture just before use).

e.g.	Reaction Solution A	100 μ l
	Reaction Solution B	3 μ l
	Reaction Solution C	2 μ l

Note:

Before covering sections with reaction solution mixture, gently shake off excess buffer left on slides from the previous step and absorb excess buffer on slides with a strip of filter paper from the edge of sections. **Do not let sections dry out!**

6. Wash sections in 0.01 M PBS 3 times, 5 minutes each.
7. Cover the entire section with **Detection Reagent** (approximately one drop for each cm² of section to be labeled) and incubate at 30°C for 30 minutes.
8. Wash sections in 0.01 M PBS 3 times, 5 minutes each.
9. Cover the entire section with **Chromogen solution*** for 5-10 minutes (approximately 100 µl for each cm² of section to be labeled).

Note:

- For the best results, pour out the chromogen solution into a test tube and bring it to room temperature before using.
- After 5 minutes of incubation, observe color development under a microscope. Stop the reaction by transferring sections into distilled water.

* Chromogen solution contains 3',3'-diaminobenzidine, which is a potential carcinogen. Avoid contact with skin. Handle with caution.

10. Wash sections in distilled water 3 times, 3 minutes each.
11. Let sections air-dry.
12. Counterstain sections with methyl green (optional).
13. Dehydrate sections in absolute alcohol for 2 minutes, clear in xylene or xylene substitutes, 2 changes, 2 minutes each, and coverslipped in resinous mounting medium (e.g. Permount®).

Permount® is a registered trademark of Fisher Scientific.

VIII. REFERENCES

1. Gavrieli Y., Sherman Y. and Ben-Sasson SA (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. *J. Cell Biol.* 119: 493-501.
2. Wyllie A. H. (1980) Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 284: 555-556.
3. Arends M. J., Morris R. G. and Wyllie A. H. (1990) Apoptosis: the role of the endonuclease. *Amer. J. Pathol.* 136: 593-608.
4. Hsu S. M., Raine L. and Fanger H. (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J. Histochem. Cytochem.* 29: 577-580.