FD NeuroTechnologies, Inc.

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FD Apop™ Kit

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

User Manual

PK 101, Version 2012-01

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

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I. Introduction

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 the most characteristic feature of apoptosis[2, 3]. The integrated biotins are amplified and visualized with the avidin-biotin-complex method[4], enabling light microscopic identification.

The reagents and procedure of the 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 and paraffin sections, as well as cultured cells.

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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	5 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:
   - Microscope slides & coverslips
   - Staining jars
   - Xylenes or xylene substitutes
   - Resinous mounting medium (e.g. Permount®)
   - A light microscope
   -

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IV. Safety and Handling Precautions

1. FD Apop™ Kit is made for *in vitro* research use only and not for drug, diagnostic or other uses.
2. The kit contains reagents that may be toxic and harmful in contact with skin or if ingested. Do not pipette by mouth. Avoid contact with skin and eyes. In case of contact, wash immediately with generous amounts of water and seek medical advice. If swallowed, wash out mouth with water and immediately call a physician.
3. Perform experiment under a chemical hood. **Wear suitable protective clothing, gloves and eye/face protection while handling kit reagents.** Wash hands thoroughly after performing the experiment.

V. Tissue Preparation
(for unfixed frozen sections)

☑ **Note**

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

1. Unfixed frozen sections* should be cut at 20 µm and be directly mounted on polylysine-coated or proteinase-resistant (Cat. #PO102) microscope slides.

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

2. Sections may be dried at room temperature for 20-30 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.

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

VI. Section Pretreatment

☑ **Note**

- *Labeling procedure (cf. page 9) should be continued once the section pretreatment commences.*
- *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 directly be transferred into the fixative solution).

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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

Sections to be labeled should be encircled with a PAP pen before fixation or after the first wash.

3. Complete labeling procedure (see page 9).

For paraffin sections:

1. Deparaffinize sections in xylene, 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.

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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 10 minutes (10x PBS provided, dilute with distilled water before use).

2. Cover the entire section with **Digestive Enzyme** (approximately 50 µl for each cm^2 of the 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, 2 minutes each.

4. Cover the entire section with **Equilibration Buffer** (approximately 100 µl for

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each cm^2 of the section to be labeled) and incubate for 5-20 minutes (in the mean-time, prepare **Reaction Solution Mixture** for the next step, see below).

5. Cover the entire section with **Reaction Solution Mixture** (approximately 20 µl for each cm^2 of the 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 µl
	Reaction Solution B	3 µl
	Reaction Solution C	2 µl

   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 cm^2 of the section to be labeled) and incubate at 30°C for 30 minutes.

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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 cm^2 of the section to be labeled).

   Note

   - *For the best result, 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®).

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