APOPTEST™-BIOTIN (cat. no.: B500)

Annexin V-Biotin Kit: *100 µl Annexin V-Biotin solution*
10 ml 10x concentrated buffer
1 vial 250 ug solid propidium iodide

Description
Annexin V-Biotin (1:8 stoichiometric complex) and 10x concentrated binding buffer.

Contents
1 vial containing 100 µl Annexin V-Biotin solution.
6 vials containing 1.7 ml 10x concentrated binding buffer.
1 vial 250 ug solid propidium iodide

Features of Annexin V-Biotin

Annexin V-Biotin	:	1:8 stoichiometric complex.
Purity	:	> 99% pure according to Fast Protein Liquid Chromatography.
Quality	:	> 99% of the protein has full phospholipid binding properties according to ellipsometry.
Quantity	:	100 µl Annexin V-Biotin solution (500 µg/ml).
Additives	:	None.

Biologival properties
Annexin V-Biotin binds to phosphatidylserine.

Storage of Annexin V-Biotin solution and 10x concentrated buffer.
Store in the dark and refrigerated at 2-8^{o}C.

Application
The APOPTEST™-BIOTIN is designed to measure quickly Apoptosis in a variety of adherent cell types in culture and in tissues *in vitro* and *in vivo*.
APOPTEST™-BIOTIN can be applied to samples derived from mammals, avian and insects.

APOPTEST™-BIOTIN is to be used *in vitro* for research purposes only, not for diagnostic or therapeutic procedures!

APOPTEST™ - BIOTIN Protocol *(B500)*

*This protocol has been devised for **APOPTEST™ - BIOTIN** staining of mouse embryos with subsequent evaluation in **Whole Mounts (WM)**, **Light Microscopy (LM) and Electron Microscopy(EM)** (reference [1])*

Materials:

- Hamilton -Syringe based pipette system with glass pipettes. Pipettes tip diameter of 15 - 25 µm.
- HEPES buffer at 37°C
- 4% Formalin/HEPES buffer at 4°C
- PBS buffer
- 0,3% Triton X 100 in PBS buffer
- **APOPTEST™ - BIOTIN (B500)**
- 0,01% proteinase K
- 1% H_2O_2 in Tris/EDTA
- horseradish peroxidase conjugated avidin with 3,3'-Diaminobenzidin tetrahydrochlorid(DAB)(0,05%) as a substrate.
- Tris/EDTA buffer at 4°C
- methanol/ H_2O_2 (9:1 v/v)
- Hematoxylin
- 2% glutaraldehyde and 2 % paraformaldehyde in 0.1 M cocadylate buffer
- 1.5% OsO_4 in 8% glucose solution
- destilled water
- 3% urany acetate
- dimethoxy propane
- Durcupan

Microinjection of mouse embryos

Inject approximately 3 µL of undiluted **APOPTEST™ - BIOTIN** solution into the ventricle of the heart using a Hamilton-Syringe based pipette system while the embryo is kept in HEPES buffer at 37°C. Temporary blanching of the umbilical vein can be observed after successful injection.
After incubation for 30 minutes the embryos are examined for heart activity. Successfully injected embryos with positive heart activity may be fixated overnight in 4% Formalin/HEPES buffer at 4°C for further processing in WM or LM evaluation.

APOPTEST™ - BIOTIN Protocol *(B500)*

Whole Mounts(WM) evaluation

After overnight fixation in 4% Formalin/HEPES buffer at 4°C the embryos are washed with PBS and with 0.3% Triton X 100 in PBS. Digestion is performed with 0.01% proteinase K for 10 minutes. Endogenous peroxidase activity is blocked by incubation with 1% H_2O_2 in Tris/EDTA for 60 minutes.
After washing with PBS the biotin labelled probe may be visualised by using the avidin-biotin complex method with horseradish peroxidase conjugated avidin with 3,3'-Diaminobenzidin tetrahydrochlorid(DAB)(0,05%) as a substrate at room temperature.
Specimens may be stored in Tris/EDTA buffer at 4°C until examination under a microscope.

Light Microscopy(LM) evaluation

After overnight fixation in 4% Formalin/HEPES buffer at 4°C embryos are dehydrated, embedded in paraffin and serially sectioned at 3 µm. Endogenous peroxidase is blocked by incubation in methanol/ H_2O_2 (9:1 v/v) for 20 minutes. Sections are washed in PBS and the biotin labelled probe is visualised by using the avidin-biotin complex method at room temperature. Sections are counterstained with Hematoxylin.

Electron Microscopy (EM) evaluation

Day 13 embryos are microinjected with **APOPTEST™ - BIOTIN (B500)** as described above (see: microinjection of mouse embryos , page 1 of the protocol). The mouse embryos are subsequently perfused intracardially with 0.5 mL 2% glutaraldehyde and 2 % paraformaldehyde in 0.1 M cocadylate buffer.
The limbs are removed , postfixed overnight in the same fixative, and cut on a Vibratome into 50 µm sections that have been processed to visualise the biotinylated probe as described for LM (see section: Light Microscopy evaluation). After the DAB reaction the sections are postfixed in 1.5% OsO_4 in a 8% glucose solution, rinsed with distilled water, stained en bloc in 3% uranyl acetate, dehydrated in dimethoxypropane, and embedded in Durcupan (reference [2]). Ultrathin sections cut on an ultratome are stained with leadcitrate are ready for examination under the Electron Microscope.

References:

1. Stefan M van den Eijnde , Antonius JM Luijsterburg, Lenard Boshart, Chris I de Zeeuw, Jan Hein van Dierendonck, Chris PM Reutelingsperger, and Christl Vermeij-Keers; Cytometry 29: 1-8 (1997)

2. De Zeeuw CI, Holstege JC, Ruigrok TJH, Voogd J
J. Comp Neurol 284: 12-35 (1989)