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Product datasheet

TUNEL Assay Kit - HRP-DAB ab206386

2 Abreviews 152 References 3 Images

Overview

Product name	TUNEL Assay Kit - HRP-DAB
Detection method	Colorimetric
Sample type	Tissue, Adherent cells
Assay type	Cell-based (qualitative)
Assay time	5h 0m
Product overview	TUNEL Assay Kit - HRP-DAB ab206386 allows the recognition of apoptotic nuclei in paraffin-embedded tissue sections, frozen tissue sections, or in preparations of single cell suspensions fixed on slides. The TUNEL assay is used to detect DNA fragmentation, such as in apoptosis. It uses terminal deoxynucleotidyl transferase (TdT) to catalyze the incorporation of deoxynucleotides at the free 3'-hydroxyl ends of fragmented DNA. The deoxynucleotides are then labeled in a variety of ways for detection of the degree of DNA fragmentation. In this TUNEL assay protocol: - terminal deoxynucleotidyl Transferase (TdT) binds to exposed 3'-OH ends of DNA fragments generated in response to apoptotic signals and catalyzes the addition of biotin-labeled deoxynucleotides - biotinylated nucleotides are bound with a streptavidin-horseradish peroxidase (HRP) conjugate - diaminobenzidine (DAB) reacts with the HRP labeled sample to generate an insoluble colored (brown) substrate at the site of DNA fragmentation - counterstaining with methyl green aids in the evaluation of normal and apoptotic cells ==
Notes	This kit is designed for chromogenic TUNEL staining with HRP and DAB. It was previously called In situ Apoptosis Detection Kit (DAB). To use FITC (Ex/Em = 495/519 nm) as a label, we recommend **TUNEL Assay Kit - FITC (ab66108)**. To use BrdU-Red (Ex/Em = 488/576nm) as a label, we recommend **TUNEL Assay Kit - BrdU-Red (ab66110)**. Find out more about the TUNEL method in the **TUNEL staining / TUNEL assay guide**. **The methyl green counterstain is water soluble and so a non-aqueous/organic mounting**

media needs to be used.

How other researchers have used HRP-DAB TUNEL Assay Kit ab206386

This TUNEL assay kit has been used in publications in a variety of sample types, including:
- Human: LX-2 cell cultures[1]
- Mouse (paraffin-embedded sections): abdominal aortic aneurysm lesion[2], thyroid gland[3], lung tissue[4], heart[5], liver[6], ovary tissue[7], skin[8], liver[9]
- Rat: paraffin embedded heart[10], brain tissue[11]
- Other: paraffin embedded human xenograft tumors in mice[12], paraffin-embedded tissue from nude mice injected with human pancreatic cancer cell line[13]

References: 1-Park SM et al 2017, 2-Li et al 2019, 3-Iglesias-Osma MC et al 2019, 4-Yuan X et al 2018, 5-Ravi V et al 2018, 6-Jadhav K et al 2018, 7-Gao Y et al 2017, 8-Shin JM et al 2017, 9-Kim MH et al 2017 and 2016, 10-Zhang J et al 2018, 11-Xiao B et al 2017, 12-Nielsen CF et al 2017, 13-Dauer et al 2019

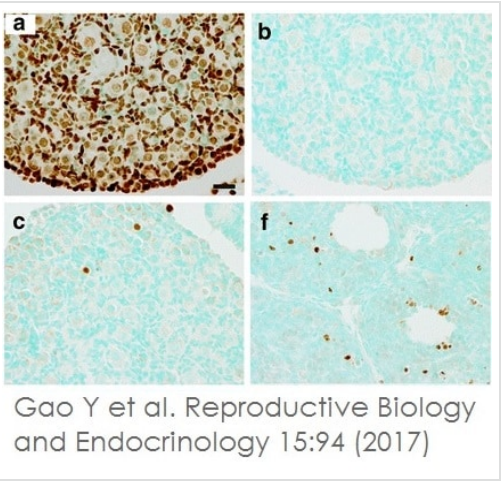
Properties

Storage instructions Store at -20°C. Please refer to protocols.

Components	30 slides	60 slides	30 slides	60 slides
25X Conjugate	1 x 150µl	1 x 300µl	1 x 150µl	1 x 300µl
Blocking Buffer	1 x 12ml	1 x 24ml	1 x 12ml	1 x 24ml
DAB Solution 1 (DAB Concentrate)	1 x 150µl	1 x 300µl	1 x 150µl	1 x 300µl
DAB Solution 2 (Substrate Reaction Buffer)	1 x 4ml	1 x 8ml	1 x 4ml	1 x 8ml
Methyl Green Counterstain	1 x 3.5ml	2 x 3.5ml	1 x 3.5ml	2 x 3.5ml
Proteinase K	1 x 50µl	1 x 100µl	1 x 50µl	1 x 100µl
Stop Buffer	1 x 4ml	1 x 8ml	1 x 4ml	1 x 8ml
TdT Enzyme	1 x 40µl	1 x 70µl	1 x 40µl	1 x 70µl
TdT Equilibration Buffer	1 x 4ml	1 x 8ml	1 x 4ml	1 x 8ml
TdT Labeling Reaction Mix	1 x 1.3ml	2 x 1.3ml	1 x 1.3ml	2 x 1.3ml

Relevance Internucleosomal DNA fragmentation is a hallmark of apoptosis in mammalian cells.

Images



TUNEL assay staining

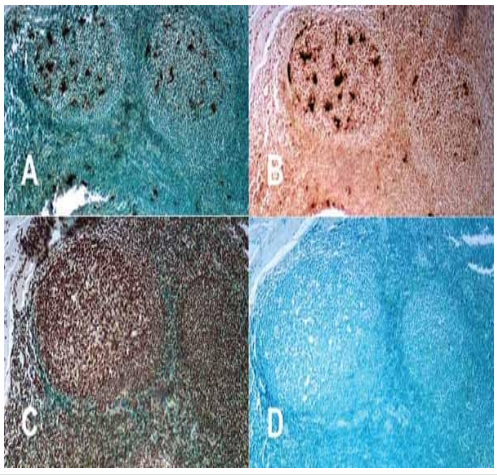
Gao Y et al used In situ Apoptosis Detection Kit / TUNEL assay ab206386 to analyze tissue sections from mouse ovaries.

a. Section treated with DNase I as positive control

b. Negative control without TdT enzyme

c and f. representative experimental images.

Nuclei stained with the TUNEL assay are brown. Sections were counter-stained with Methyl Green.



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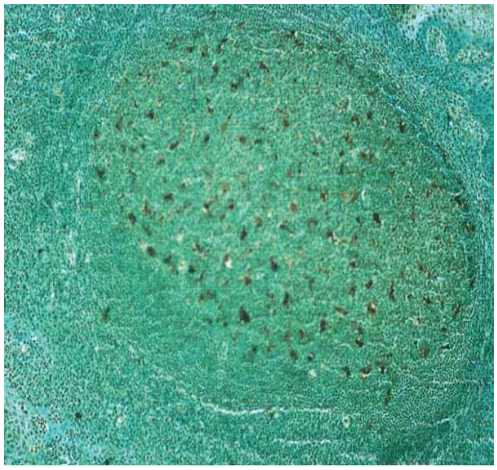
Using paraffin fixed human tonsil tissue,10 µm sections (1000X). A] Section processed and counter-stained with methyl green according to the manual. B] Counter-stain step was eliminated to more clearly illustrate the level of positive staining in the germinal centres of tonsil tissue. C] Section treated with DNase I in order to generate a positive control slide. Note all nuclei stain positive. The use of DNase I generates free 3'-OH groups on cellular DNA, these free 3'-OH groups are then labelled with biotin-nucleotide by the TdT in the kit. D] Negative control, the TdT enzyme step was eliminated thereby generating a negative slide.



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Using paraffin fixed human tonsil tissue, 10 µm sections (1000X)

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