Product Information

ApoBrdU-IHC DNA Fragmentation Assay Kit

I. Kit Contents:

Components	K2072-50 50 assays	Cap Color	Store Temp.	Part Number
Control Slides-pos/neg	2 ea	natural box	-20℃	K2072-C-1
Blocking Buffer	22 ml	white cap	-20℃	K2072-C-2
H_2O_2/Urea Tablets	6 ea	amber vial	-20℃	K2072-C-3
Protease K	11 ml	pink cap	-20℃	K2072-C-4
DAB Tablets	6 ea	amber vial	-20℃	K2072-C-5
TdT Enzymes	0.041 ml	yellow cap	-20℃	K2072-C-6
Br-dUTP	0.44 ml	violet cap	+4℃	K2072-C-7
200X Conjugate	0.035 ml	clear cap	+4℃	K2072-C-8
5X Reaction Buffer	1.75 ml	green cap	-20℃	K2072-C-9
Anti-BrdU-Biotin Antibody	0.275 ml	orange cap	-20℃	K2072-C-10
Methyl Green	6 ml	natural cap	room temp.	K2072-C-11

II. Introduction:

Internucleosomal DNA fragmentation is a sign of apoptosis in mammalian cells. The ApoBrdU-IHC DNA Fragmentation Assay Kit provides a simple and convenient way for detection of DNA fragmentation in fixed cell preparations or tissue sections based on immunohistochemistry method. The assay provides a two-color TUNEL (Terminal deoxynucleotide transferase dUTP Nick End Labeling) assay to label DNA fragmentation and then detect apoptotic cells by immunohistochemistry. The kit provides complete components including positive and negative control slides for assessing reagent performance; terminal deoxynucleotidyl tranferase enzyme (TdT), proteinase K, bromodeoxyuridine triphosphate (Br-dUTP), biotin labeled anti-BrdU antibody for labeling DNA fragmentation; reaction and blocking buffers for processing individual steps; horseradish peroxidase streptavidin conjugate, H_2O_2/Urea Tablets, DAB Tablets for color production and methyl green solution for counter staining the cells.

III. General Consideration:

The components of this kit are for Research Use Only. To avoid reagent loss, centrifuge vials before using.

After initial defrosting, the 5X Reaction Buffer and Anti-BrdU-Biotin Antibody should be stored at 4℃, and Methyl Green should be stored at room temperature. Do Not Refreeze.

The control slides contain a mixture of apoptotic and non-apoptotic cells, allowing visualization of both positive & negative labeling within the same microscope field.

Incubation time for proteinase K, DNase I, and the end labeling of the DNA may need to be empirically determined for your particular cell type and slide preparation. Use this protocol as a starting guideline.

IV. Assay Protocol: Staining of Paraffin Embedded Tissue (PET):

PET-A. Deparaffinization & Rehydration

1. Immerse slides in xylene for 5 minutes at room temperature. Repeat using fresh xylene for second 5 minutes incubation.

2. Immerse slides in 100% ethanol for 5 minutes at room temperature. Repeat using fresh 100% ethanol for second 5 minutes.

3. Immerse slides in 90% ethanol for 3 min, then 80% ethanol for 3 min, and then 70% ethanol for 3 minutes at room temperature.

4. Immerse slides briefly into 1X PBS and carefully dry the glass slide around the specimen.

If processing the kit's control slides simultaneously with unknown samples, please refer to the CFS protocol (CSF-A).

At this point it may be helpful to encircle the specimen using a waxed pen or a hydrophobic marker.

Do not let tissue specimen dry out at any step! If necessary, cover or immerse specimen in 1X PBS to keep hydrated!

PET-B. Permeabilization, Inactivation of Endogenous Peroxidase, & Equlibration

5. Dilute only enough Proteinase K (pink cap) needed 1:100 in 10 mM Tris pH 8. Cover the entire specimen with 100 µl proteinase K. Incubate at room temperature for 20 minutes. DO not over incubate.

6. Rinse slide with 1X PBS. Gently tap off excess liquid and carefully dry the glass slide around the specimen.

7. Dilute 30% H_2O_2 1:10 in methanol. Cover the entire specimen with 100 µl of 3% H_2O_2. Incubate at room temperature for 5 minutes. DO not over incubate.

8. Rinse slide with 1X PBS. Gently tap off excess liquid and carefully dry the glass slide around the specimen.

9. Dilute only enough 5X Reaction Buffer (green cap) as needed 1:5 with dH_2O. Cover the entire specimen with 100 µl of the 1X Reaction Buffer. Incubate at room temperature for 10 to 30 minutes while preparing the labeling reaction mixture below.

PET-C. End Labeling Reaction & Detection

10. Prepare the Complete Labeling Reaction Mixture as follows (Note: Mix only enough DNA Labeling Solution to complete the number of assays prepared per session. The DNA Labeling Solution is active for approximately 24 hours.)

DNA Labeling Solution	1 assay	5 assays	10 assays
5X Reaction Buffer (green cap)	10 µl	50 µl	100 µl
TdT Enzyme (yellow cap)	0.75 µl	3.75 µl	7.5 µl
Br-dUTP (violet cap)	8 µl	40 µl	80 µl
Distilled H_20	32.25 µl	161.25 µl	322.5 µl
Total Volume	51 µl	255 µl	510 µl

11. Carefully blot the 1X Reaction Buffer from the specimen, taking care not to touch the specimen. Immediately apply 50 µl of Complete Labeling Reaction Mixture (prepared above) onto each specimen except for the control slides which require only 25 µl each (Note: The use of a cover slip at this point assures even distribution of the reaction mixture and prevents evaporation during incubation).

12. Cover the specimen with a piece of Parafilm cut slightly larger than the specimen (HINT: Folding up one corner of the Parafilm cover slip will aid in its application and removal). Place slides in a humid chamber and incubate at 37℃ for 1 to 1.5 hours.

Note: The DNA End Labeling Reaction can also be carried out at 22 - 24℃ overnight for the control slides. For samples other than the control slides provided in the kit, incubation times at 37℃ may need to be adjusted to longer or shorter periods depending on the characteristics of the tissue you used.

13. Remove Parafilm cover slip and rinse slide with PBS. Gently tap off excess liquid and carefully dry the glass around the specimen.

14. Cover the entire specimen with 100 µl of Blocking Buffer (white cap). Incubate at room temperature for 10 minutes. Carefully blot the Blocking Buffer from the specimen, taking care not to touch the specimen.

15. Immediately cover specimen with 100 µl of Antibody Solution (prepared as described below).

Antibody Solution	1 assay	5 assays	10 assays
Anti-BrdU-Biotin Antibody (orange cap)	5 µl	25 µl	50 µl
Blocking Buffer (white cap)	95 µl	475 µl	950 µl
Total Volume	100 µl	500 µl	1000 µl

16. Incubate with the Antibody Solution in the dark for 1 - 1.5 hours at room temperature (Hint: Cover slides with aluminum foil).

17. Rinse slide in PBS. Gently tap off excess liquid and carefully dry the glass around the specimen. Cover the entire specimen with 100 µl of Blocking Buffer (white cap).

18. Dilute only enough of the 200X Conjugate (black cap) needed 1:200 in Blocking Buffer (white cap). Prepared as described below.

Conjugate Solution	1 assay	5 assays	10 assays
200X Conjugate (black cap)	0.5 µl	2.5 µl	5.0 µl
Blocking Buffer (white cap)	100 µl	500 µl	1000 µl

19. Carefully blot the Blocking Buffer from the specimen, taking care not to touch the specimen. Immediately apply 100 µl of diluted conjugate to the specimen. Incubate at room temperature for 30 minutes.

20. Five minutes before concluding incubation prepare DAB solution by dissolving one tablet of DAB (amber vial) and one tablet of H_2O_2/Urea (amber vial) in one ml of tap H_2O. This yields enough DAB solution for 10 specimens. (Note: Tap H_2O may contain metal ions that enhance the DAB reaction. DAB is highly carcinogenic and care should be taken when handling).

21. Rinse slides with 1X PBS. Gently tap off excess liquid and carefully dry the glass slide around the specimen. Cover the entire specimen with 100 µl of DAB solution. Incubate at room temperature for 15 minutes. Rinse slides with H_2O and blot.

PET-D. Counterstain

22. Immediately cover the entire specimen with 100 µl of Methyl Green Counterstain (natural cap) solution. Incubate at room temperature for 3 minutes. Press edge of the slide against an absorbent towel to draw off most of the counterstain and place in a coplin jar slide holder.

23. Dip slides 2 times briefly into 100% ethanol. Blot slides briefly on an absorbent towel. Repeat step 22 using fresh 100% ethanol.

24. Blot slides briefly on an absorbent towel. Dip slides into xylene (or xylene substitute). Wipe excess xylene from back of slide and around specimen.10. Mount a glass cover slip using a mounting media such as permount (r) over the specimen.

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For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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