KAMIYA BIOMEDICAL COMPANY

BrdU IHC Kit

For the detection and localization of bromodeoxyuridine incorporated into newly synthesized DNA of actively proliferating cells

Cat. No. KT-077

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

BrdU IHC Kit
Cat. No. KT-077

PRODUCT
The **K-*ASSAY*®** BrdU IHC (immunohistochemistry) Kit is for the detection and localization of bromodeoxyuridine (BrdU) incorporated into newly synthesized DNA of actively proliferating cells. For research use only. Not for use in diagnostic procedures.

PRINCIPLE
A non-isotopic immunohistochemical staining procedure for the localization of DNA synthesis and cell proliferation.

Evaluation of cell cycle progression is essential for investigations in many scientific fields. Measurement of [^{3}H] thymidine incorporation as cells enter S phase has long been the traditional method for the detection of cell proliferation. Subsequent quantification of [^{3}H] thymidine is performed by scintillation counting or autoradiography. This technique is slow, labor-intensive and has several limitations including the handling and disposal of radioisotopes and the necessity of expensive equipment.

A well-established alternative to [^{3}H] thymidine uptake has been demonstrated by numerous investigators. In these methods BrdU, a thymidine analog, is incorporated into newly synthesized DNA strands of actively proliferating cells. Following partial denaturation of double stranded DNA, BrdU is detected immunochemically allowing the assessment of the population of cells which are actively synthesizing DNA.

The **K-*ASSAY*®** BrdU IHC Kit involves incorporation of BrdU into proliferating cells, *in vivo* or *in vitro*, and visual staining (dark brown nuclei) of these cells which is achieved using a Prediluted Biotinylated Sheep anti-BrdU Detector Antibody followed by Streptavidin-HRP Conjugate and diaminobenzidine (DAB) substrate.

COMPONENTS
- **Trypsin Enzyme Conc., 4x — 3 mL
- **Trypsin Dilution Buffer — 12 mL
- Denaturing Solution — 6 mL
- Blocking Buffer — 6 mL
- Detector Antibody (biotinylated & prediluted) — 6 mL
- Streptavidin-HRP Conjugate — 6 mL
- Substrate Reaction Buffer — 6 mL
- DAB Concentrate — 0.3 mL
- ***Hematoxylin Counterstain — 6 mL
- Mounting Media — 6 mL
- 5 Control Slides: Fixed cells labeled with BrdU
  - 1 x Positively stained
  - 4 x Unstained

The material in this kit is sufficient to run 50 slides. The average test area is defined as a circle around the tissue with an approximate diameter of 2 cm.

**Trypsin is only required if using formalin fixed tissues. If the tissues are fixed in alcohol, trypsin digestion is not required.

***Filter before use and protect from light. If desired, dilute 1:5 with distilled water immediately prior to use for less intense staining.

Materials or equipment required but not provided
- Hydrogen peroxide (30% solution) for quenching endogenous peroxidase activity

- Phosphate buffered saline (PBS) solution
- Distilled water
- Ethyl alcohol
- Xylene
- Coverslips
- Bromodeoxyuridine (BrdU)
- Methanol

PREPARATION OF SLIDES

Paraffin-Embedded tissue sections:

1. Sample animals are labeled with BrdU.
2. Animals are sacrificed by inhalation of isofluorane and perfused with PBS followed by 4% buffered formalin.
3. Target tissue is removed and immersed in 4% buffered formalin overnight.
4. Tissue is then dehydrated and embedded in paraffin
    a. PBS – 10 min
    b. 70% EtOH – 1 hour
    c. 85% EtOH – 1 hour
    d. 95% EtOH – 30 min
    e. 100% EtOH – 15 min (2X)
    f. Xylene – 15 min (2X)
    g. 1:1 Xylene and Paraffin – 45 min
    h. Paraffin – 30 min (4X)
5. 5 micron sections are cut from the paraffin blocks and placed on slides.
6. Slides remain on a 37°C heating tray overnight and are then stored at 4°C.

Cultured Cells and Cell Suspensions

Preparation of Cells

A. Cells in Flasks

1. Using sterile tissue culture techniques, culture cells with 10 μM BrdU for 2-24 hours at 37°C.
2. Remove the media containing the BrdU label and wash twice with PBS.
3. Using a cytospin, centrifuge 100 μL of cells at 1 x 10^6 cells/mL onto suitable slides and allow to air dry.

B. Cells on Chamber Slides (Adherent cells only)

1. Using sterile tissue culture techniques, culture cells in chambers with 10 μM BrdU for 2-24 hours at 37°C.
2. Remove the labeling media and wash three times with PBS for about 5 seconds per wash.
3. Remove the chamber.
4. Fix cells with 4% formaldehyde for 30 minutes.
5. Wash three times with PBS in a coplin jar.
6. Fix and permeabilize cells with 70% ethanol for 30 minutes.
7. Dehydrate cells with ascending (80%, 90%, 100%) ethanol series in a coplin jar, 30 seconds each.
8. Let slides dry, then proceed with Staining Protocol.

STAINING PROTOCOL

1. Deparaffinization (FOR PARAFFIN-EMBEDDED TISSUES ONLY)

*Note: **If you are not using paraffin-embedded tissues, skip to step 2 below.** If paraffin-embedded tissues are used, it is necessary to deparaffinize the slides before following the BrdU staining protocol below.*

Deparaffinization involves incubation of the slides in xylene followed by a graded alcohol series as follows:

Xylene	5 minutes, then change to new coplin jar containing xylene
Xylene	5 minutes
100% ethyl alcohol	5 minutes
90% ethyl alcohol	3 minutes
80% ethyl alcohol	3 minutes
70% ethyl alcohol	3 minutes
PBS	3 minutes

2. Staining

Component / Preparation	Procedure	Time (Min.)
Hydrogen peroxide *(not provided)*: Quenching solution. Dilute 30% hydrogen peroxide* 1:10 in methanol.	Immerse slides into a coplin jar or other appropriate container filled with quenching solution for 10 minutes. Wash in PBS 1x for 2 minutes.	10
4x Trypsin Enzyme Concentrate and Trypsin Dilution Buffer: Trypsin (0.2% solution)** FOR FORMALIN-FIXED TISSUES ONLY. Add 1 drop of 4x Trypsin Enzyme Concentrate to 3 drops of Trypsin Dilution Buffer and mix well.	Add 2 or more drops to each slide. Incubate at room temperature for 10 minutes, followed by a 3 minute rinse in distilled water.	10
Denaturing Solution	Add 2 or more drops to each slide and incubate at room temperature for 30 minutes. Wash twice with PBS, 2 minutes per wash.	30
Blocking Buffer	Add 2 or more drops to each slide and incubate at room temperature for 10 minutes. Drain the solution by blotting on paper towels (DO NOT RINSE.)	10
Detector Antibody	Add 2 or more drops to each section and incubate at room temperature for 60 minutes. Wash twice with PBS, 2 minutes per wash.	60
Streptavidin-HRP Conjugate	Add 2 drops or more to each section and incubate at room temperature for 10 minutes. Wash twice with PBS, 2 minutes per wash.	10
Substrate Reaction Buffer and DAB concentrate	Add 1 µL DAB concentrate for every 29 µL Substrate Reaction Buffer (assume approximately 100 µL/slide). For 10 slides, this works out to be 1 drop of DAB concentrate to 1 mL of Substrate Reaction Buffer. Mix well and add 2 or more drops per slide and incubate at room temperature for 10 minutes. Wash with distilled water for 2 minutes.	10
Hematoxylin Counterstain (Filter before use. For less intense staining, dilute 1:5 with distilled water immediately before use)	Add 2 or more drops of hematoxylin per slide and incubate at room temperature for 1-5 minutes. Wash slides briefly with tap water. Incubate slides for 1 minute in PBS until color turns blue. Give a final two-minute wash in distilled water.	1-5
Mounting Media	Incubate slides in 90% ethanol for 30 seconds, 100% ethanol for 30 seconds and xylene for 30 seconds (2 times each). Add 1-2 drops of mounting media and coverslip.	

* Hydrogen peroxide is not stable for long periods of time. Be sure the reagent you are using has not expired.

** The concentration of trypsin used is very important. It may be necessary to titer the trypsin reagent for use in your system. Usually a final concentration of 0.02% to 0.2% is appropriate. *Other methods for digesting the tissue to expose epitopes for antibody recognition may also be used.*

SAMPLE PICTURES

Example picture of formalin-fixed MCF7 cells labeled with BrdU stained using BrdU IHC kit. More diffuse nuclear immunostaining (Figure 1) can be seen next to a more localized nuclear staining reaction (Figure 2).

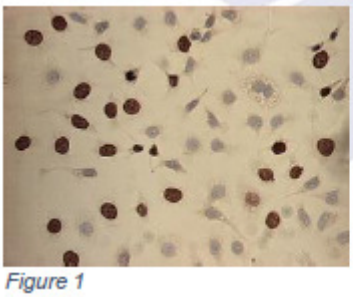

Figure 1

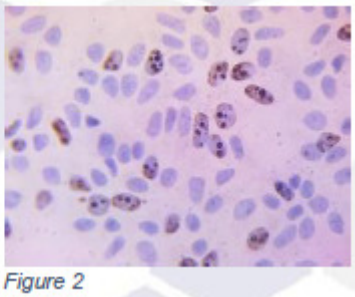

Figure 2

STORAGE

Upon receipt, store the entire kit at -20℃. Once the kit is thawed, you may keep at 4℃ for 5 days. For long term storage, it is recommended you aliquot and freeze the components at -20℃, particularly the Streptavidin-HRP Conjugate, the Detector Antibody, the 4x Trypsin Concentrate and the control slides.

WARNINGS AND PRECAUTIONS

Wearing of latex or rubber gloves, a lab coat and safety goggles is recommended when running this kit; especially avoid inhalation of DAB reagent and contact with skin and clothes.

TROUBLESHOOTING

Poor Positive Staining or No Positive Staining with Little or No Background Staining:

1. Little or no BrdU labeling occurred in the tissue or cells prior to preparing the slides.
2. Detector Antibody or Streptavidin-HRP reagent was omitted or used in the wrong order.
3. Use a longer incubation time for Detector Antibody.
4. Use a longer incubation time for DAB Substrate (view slide while it is developing).
5. Since excessive counterstaining can compromise positive brown DAB staining, try using shorter Hematoxylin Counterstain incubation time.
6. DO NOT LET SLIDES DRY OUT; Keep wet at all times during the staining procedure.
7. Insufficient blotting between blocking step and Detector Antibody step. This could dilute out the Detector Antibody component.
8. If tissue is formalin-fixed and digestion of the tissue is necessary, the trypsin component may need titering.
9. Use fresh xylene solution, as solution which has been used many times will contain residual paraffin and may interfere with staining.

High Background Staining:

1. Reduce substrate incubation time.
2. Check to make sure the substrate-DAB reagent was prepared correctly (the right ratio of DAB concentrate to Substrate Reaction Buffer).
3. Reduce concentration of the Streptavidin-HRP component.
4. Increase the number and time of washes in-between steps.
5. Slides incorrectly deparaffinized (use fresh reagents, xylene and ethanol, for the deparaffinization procedure).
6. Try longer incubation during the blocking step.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com