



Exalpa
biologicals INC

BrdU Immunohistochemistry Kit Instruction Manual

Features

- Easy to use system
- Reagents titered for success
- Proven protocol

Ordering Information

Catalog Number
X1545K.1

Size
50 Slides

Format
Immunohistochemistry Kit

Species Reactivity
Ubiquitous

Company Information
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Other kits & Reagents Available from Exalpha Biologicals

DNA Fragmentation Detection Kit

X2044K1 (30 Tests)

X2044K2 (60 Tests)

BrdU Cell Proliferation Assay Kit

X1327K1 (200 Tests)

X1327K2 (1000 Tests)

X1327K3 (5000 Tests)

BrdU Chemiluminescent Cell Proliferation Assay Kit

X1623K1 (200 Tests)

X1623K2 (1000 Tests)

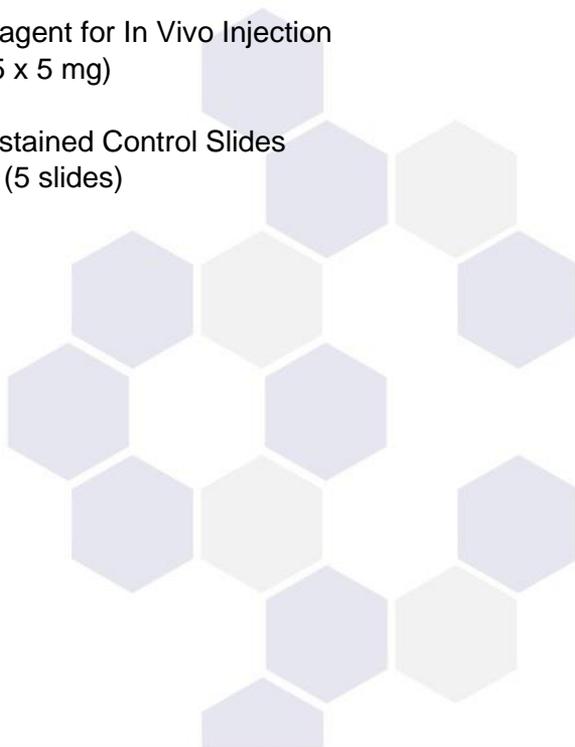
X1623K3 (5000 Tests)

BrdU Reagent for In Vivo Injection

X2834 (5 x 5 mg)

BrdU Unstained Control Slides

X2743.1 (5 slides)





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Intended Use

The Exalpha Biologicals BrdU Immunohistochemistry Kit is a histochemical staining kit for the detection and localization of bromodeoxyuridine incorporated into newly synthesized DNA of actively proliferating cells.

This assay is for research use only and not for use in diagnostic or therapeutic procedures.

Storage of Kit Components

The Exalpha Biologicals BrdU Immunohistochemistry Kit components are shipped on blue ice. Upon receipt, store entire kit at -20°C . Once the kit is thawed, you may keep it at 4°C for 5 days. For long-term storage, it is recommended you aliquot and freeze the components at -20°C , particularly the Streptavidin-HRP Conjugate, the Prediluted Biotinylated Sheep anti-BrdU Detector Antibody, the 4x Trypsin Concentrate and the control slides. 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.

Background and Principles of Assay

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

Evaluation of cell cycle progression is essential for investigations in many scientific fields. Measurement of $[^3\text{H}]$ thymidine incorporation as cells enter S phase has long been the traditional method for the detection of cell proliferation. Subsequent quantification of $[^3\text{H}]$ thymidine is performed by scintillation counting or autoradiography. This technology 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 [³H] thymidine uptake has been demonstrated by numerous investigators. In these methods bromodeoxyuridine (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.

Exalpha Biologicals BrdU Immunohistochemistry 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 DAB (diaminobenzidine) substrate.

Materials Provided*

The following is the list of components which are included in the BrdU Immunohistochemistry Kit.

- 1A.** 4x Trypsin Concentrate
(Part# J0023): 3 ml.
- 1B.** Trypsin Dilution Buffer
(Part# J0020): 12 ml.
2. Denaturing Solution
(Part# J0025): 6 ml.
3. Block Buffer
(Part# J0024): 6 ml.
4. Prediluted Biotinylated Sheep anti-BrdU Detector Antibody
(Part# J0021): 6 ml.
5. Streptavidin-HRP Conjugate
(Part# J0022): 6 ml.
- 6A. DAB Substrate Reaction Buffer
(Part# J0026): 6 ml.
- 6B. DAB Concentrate
(Part# J0027): 0.3 ml.
- 7.*** Hematoxylin
(Part# J0028): 6 ml.



8. Krystalon Mounting Media
(Part# J0029): 6 ml.
9. 5 Control Slides: Formalin fixed MCF7 cells labeled with BrdU (Part# J0169): 1 Positively Stained + 4 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 centimeters.*

*** 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 Required but Not Provided

1. Hydrogen peroxide (30% solution) for quenching endogenous peroxidase activity.
2. Methanol.
3. Phosphate buffered saline (PBS) solution (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na₂HPO₄·7H₂O, 1.4 mM KH₂PO₄).
4. Distilled water.
5. Ethyl alcohol.
6. Xylene.
7. Coverslips.
8. Bromodeoxyuridine (BrdU).

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 μ M 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 μ l of cells at 1 x 10⁶ 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 μ M 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	Component name and preparation	Procedure	Time (min)
Quenching Solution (not provided)	Quenching Solution (not provided). 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 with PBS 1x for 2 minutes.	10
Components 1A and 1B	Trypsin (0.2% solution)** FOR FORMALIN FIXED TISSUES ONLY. Add 1 drop of Component 1A to 3 drops of Component 1B 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



Component	Component name and preparation	Procedure	Time (min)
Component 2	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
Component 3	Block Buffer	Add 2 or more drops to each slide and incubate at room temperature for 10 mins. Drain the solution by blotting on paper towels (DO NOT RINSE).	10
Component 4	Prediluted Biotinylated Sheep anti-BrdU 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
Component 5	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
Components 6A and 6B	DAB Substrate Reaction Buffer and DAB Concentrate	Add 1 μ l DAB concentrate for every 29 μ l Substrate Reaction Buffer (assume approximately 100 μ l/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

Component	Component name and preparation	Procedure	Time (min)
Component 7	Hematoxylin (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
Component 8	Krystalon 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 Krystalon Mounting Media and then 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.*

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. Prediluted Biotinylated Sheep anti-BrdU Detector Antibody or Streptavidin-HRP Conjugate was omitted or used in the wrong order.
3. Use a longer incubation time for Prediluted Biotinylated Sheep anti-BrdU 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 Prediluted Biotinylated Sheep anti-BrdU 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 Conjugate 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.

Sample Pictures

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

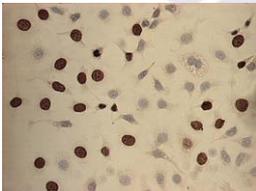


Figure 1

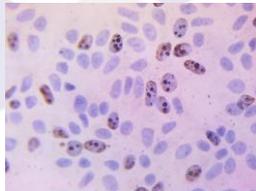


Figure 2



Ordering Information

Catalog Number	Size
X1545K.1	50 Slides

Contact Information

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