Bromodeoxyuridine (BrdU)
Immunohistochemistry kit Instruction Manual

Features

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

Ordering Information

Catalog Number
X1545K

Size
50 Slides

Format
Immunohistochemistry Kit

Species Reactivity
Ubiquitous

Company Information
Exalpha Biologicals, Inc.
tel: 800.395.1137
978.425.1370
fax: 866.924.5100
978.425.1376
info@exalpha.com
www.exalpha.com

Other ELISA Kits Available from Exalpha Biologicals

BrdU Cell Proliferation ELISA Kit

200 tests (Cat. No. X1327K1)

1000 tests (Cat. No. X1327K2)

5000 tests (Cat. No. X1327K3)

BrdU Immunohistochemistry Kit

50 sections (Cat. No. X1545K)

PIG3 (p53 Inducible Gene-3) ELISA Kit

1 Plate (Cat. No. X1326K1)

2 Plates (Cat. No. X1326K2)

Mouse TNF ELISA Kit

1 Plate (Cat. No. X1851K)

Mouse IL-17 ELISA Kit

1 Plate (Cat. No. X2748K)

Exalpha Biologicals, Inc. I 2 Shaker Rd. Bldg. B101 I Shirley, MA 01464
Tel: 800.395.1137 I Fax: 866.924.5100 I info@exalpha.com
www.exalpha.com

Table of Contents

BrdU

Immunohistochemistry Kit

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 -20ºC. Once the kit is thawed, you may keep at 4ºC for 5 days. For long-term storage, it is recommended you aliquot and freeze the components at –20ºC, particularly the Streptavidin-HRP Conjugate, the Detector Antibody and the Trypsin Concentrate. Wearing of latex or rubber gloves is recommended when running this kit; especially avoid contact of DAB reagent with skin and clothes.

Background

A non-isotopic immunohistochemical staining 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 technology is slow, labor intensive and has several limitations including the handling and disposal of radioisotopes and the necessity of expensive equipment.

Exalpha Biologicals, Inc. I 2 Shaker Rd. Bldg. B101 I Shirley, MA 01464
Tel: 800.395.1137 I Fax: 866.924.5100 I info@exalpha.com
www.exalpha.com

A well-established alternative to [^{3}H] thymidine uptake has been demonstrated by numerous investigators. In these methods bromodeoxyuridine (BrdU), a thymidine analog, replaces [^{3}H] thymidine. BrdU 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 biotinylated anti-BrdU 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 Enzyme Concentrate	
1B**	Trypsin Dilution Buffer	12 ml
2	Denaturing Solution	6 ml
3	Blocking Buffer	6 ml
4	Detector Antibody (Biotinylated and pre-diluted)	6 ml
5	Streptavidin-HRP Conjugate (pre-diluted)	6 ml
6A	Substrate Reaction Buffer	6 ml
6B	DAB Concentrate	0.3 ml
7	Hematoxylin Counterstain	6 ml
8	Mounting Media	6 ml
9	5 Control Slides: Intestinal tissue from mouse injected with BrdU	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.*

Materials Required But Not Provided

10 Hydrogen peroxide (30% solution) for quenching endogenous peroxidase activity
11 Phosphate buffered saline (PBS) solution
12 Distilled water
13 Ethyl alcohol
14 Xylene
15 Coverslips
16 Bromodeoxyuridine (BrdU)
17 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 over night.
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 – 15min (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 37ºC heating tray overnight and are then stored at 4ºC.

Cultured Cells and Cell Suspensions
Preparation of Cells

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

Exalpha Biologicals, Inc. I 2 Shaker Rd. Bldg. B101 I Shirley, MA 01464
Tel: 800.395.1137 I Fax: 866.924.5100 I info@exalpha.com
www.exalpha.com

B. Cells on Chamber Slides (Adherent cells only)

1. Using sterile tissue culture techniques, culture cells in chambers with 10μM BrdU for 2-24 hours at 37ºC.
2. Remove the labeling media and wash twice with PBS.
3. Fix cells with 70% ethanol or other suitable fixative for 30 minutes.
4. Wash twice with PBS.

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 Preparation	Procedure	Time (min)
Component 10 (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

Component 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 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	Blocking solution	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	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

Exalpha Biologicals, Inc. I 2 Shaker Rd. Bldg. B101 I Shirley, MA 01464
Tel: 800.395.1137 I Fax: 866.924.5100 I info@exalpha.com
www.exalpha.com

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
Component 6A and 6B	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
Component 7	Hematoxylin Counterstain	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	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.*

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 times for Detector Antibody.
4. Use a longer incubation time for 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

Exalpha Biologicals, Inc. I 2 Shaker Rd. Bldg. B101 I Shirley, MA 01464
Tel: 800.395.1137 I Fax: 866.924.5100 I info@exalpha.com
www.exalpha.com

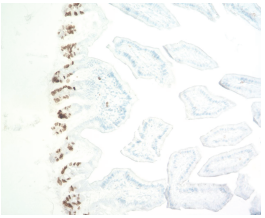
and may interfere with staining.

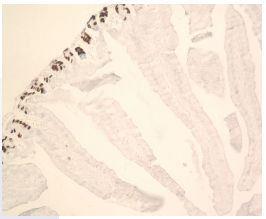
High Background Staining

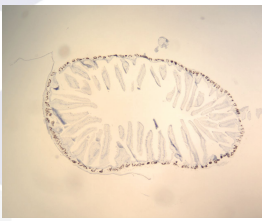
1. Reduce substrate incubation time.
2. Check to make sure the substrate-DAB reagent was prepared correctly (the right ratio of DAB concentrate to Substrate Reaction Buffer).
3. Reduce concentration of the Streptavidin-HRP Component.
4. Increase the number and time of washes in between steps.
5. Slides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

Sample Pictures

Examples pictures of formalin-fixed, paraffin embedded mouse intestinal tissue sections stained using Exalpha's BrdU Immunohistochemistry kit.







Ordering Information

Catalog Number	Size
X1545K	50 Slides

Contact Information

Exalpha Biological, Inc.
2 Shaker Road, Bldg. B101
Shirley, MA 01464

Telephone: 978.425.1370
Free Phone: 800.395.1137
Fax Number: 978.425.1376
Free Fax: 866.924.5100
url: www.exalpha.com
email: info@exalpha.com