ab125306 - BrdU Immunohistochemistry Kit

Instructions for Use

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

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

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.

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.

ab125306 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.

2. Assay Summary

Prepare slides

Deparaffinization (for paraffin-embedded tissues only), proceed to next step for non-paraffin-embedded tissues.

Incubation with Quenching Solution followed by PBS wash step.

Incubation with Trypsin Enzyme Concentrate and Dilution Buffer followed by a rinse with distilled water.

Incubation with Denaturing Solution followed by PBS wash step.

Incubation with Blocking Solution.

Incubation with Detector Antibody followed by PBS wash step.

Incubation with Streptavidin-HRP Conjugate followed by PBS wash step.

Incubation with DAB Solution 2 and DAB Solution 1 followed by distilled water wash step.

Incubation with Hematoxylin Counterstain followed by water and PBS wash steps.

Incubation with ethanol followed by xylene.

Add mounting media and coverslip.

3. Kit Contents

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.

Item	Quantity
4X Trypsin Enzyme Concentrate*	3 mL
Trypsin Dilution Buffer*	12 mL
Denaturing Solution	6 mL
Blocking Buffer	6 mL
Detector Antibody (Biotinylated and pre-diluted)	6 mL
Streptavidin-HRP Conjugate (pre-diluted)	6 mL
DAB Solution 2	6 mL
DAB Solution 1	0.3 mL
Hematoxylin Counterstain	6 mL
Mounting Media	6 mL
Control Slides (stained)**	1

Control Slides (unstained)**	4

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

** Formalin fixed MCF7 cells labeled with BrdU

4. Storage and Handling

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

5. Additional Materials Required

- Quenching Solution: Hydrogen peroxide (30% solution) for quenching endogenous peroxidase activity.
- Phosphate buffered saline (PBS) solution
- Distilled water
- Ethyl alcohol
- Xylene
- Coverslips
- Bromodeoxyuridine (BrdU)
- Methanol

6. Preparation of Slides

Paraffin-Emmbedded 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 — 15min (2X)
    f. Xylene — 15 min (2X)
    g. 1:1 Xylene and Paraffin — 45 min
    h. Paraffin — 30 min (4X)
5. Five 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 37ºC.

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.

4. Proceed with Staining Protocol.

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 labelling media and wash twice with PBS.

3. Fix cells with 70% ethanol or other suitable fixative for 30 minutes.

4. Wash twice with PBS.

5. Proceed with Staining Protocol.

7. 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*:*

Step	Substance	Incubation Time
1.1	Xylene	5 minutes, then change to new coplin jar containing Xylene.
1.2	Xylene	5 minutes
1.3	100% ethyl alcohol	5 minutes
1.4	90% ethyl alcohol	3 minutes
1.5	80% ethyl alcohol	3 minutes
1.6	70% ethyl alcohol	3 minutes
1.7	PBS	3 minutes

2. **Staining**

Step	Component	Component Preparation	Procedure	Time (Min)
2.1	Hydrogen peroxide (30% solution)	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
2.2	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 mins followed by a 3 min rinse in distilled water.	10

2.3	Denaturing Solution	N/A	Add 2 or more drops to each slide and incubate at room temperature for 30 mins. Wash twice with PBS, 2 mins per wash.	30
2.3	Blocking Buffer	N/A	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
2.4	Detector Antibody	N/A	Add 2 or more drops to each slide and incubate at room temperature for 60 mins. Wash twice with PBS, 2 mins per wash.	60
2.5	Streptavidin-HRP Conjugate	N/A	Add 2 or more drops to each slide and incubate at room temperature for 10 mins. Wash twice with PBS, 2 mins per wash.	10

2.6	Substrate Reaction Buffer and DAB concentrate	N/A	Add 1 µL DAB Solution 1 for every 29 µL DAB Solution 2 (assume approximately 100 µL/slide). For 10 slides, this works out to be 1 drop of DAB Solution 1 to 1 mL of DAB Solution 2. Mix well and add 2 or more drops per slide and incubate at room temperature for 10 mins. Wash with distilled water for 2 mins.	10
2.7	Hematoxylin Counterstain	N/A	Add 2 or more drops of hematoxylin per slide and incubate at room temperature for 1-5 mins. Wash slides briefly with tap water. Incubate slides for 1 min in PBS until color turns blue. Give a final 2-min wash in distilled water.	1 - 5

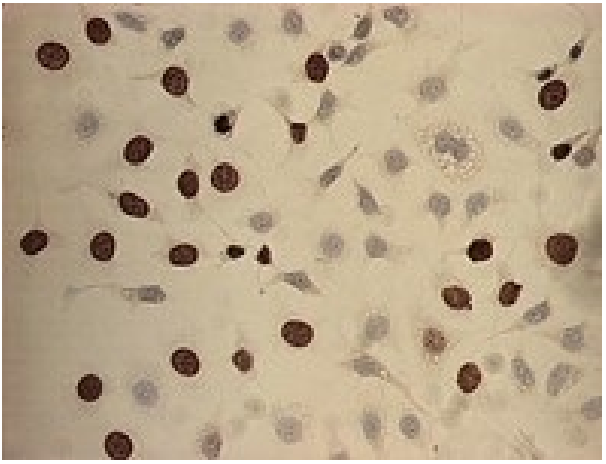
2.8	Mounting Media	N/A	Incubate slides in 90% ethanol for 30 seconds, 100% ethanol for 30 Seconds and xylene for 30 seconds (2 times each). Add 1-2 drops of mounting media and coverslip.	

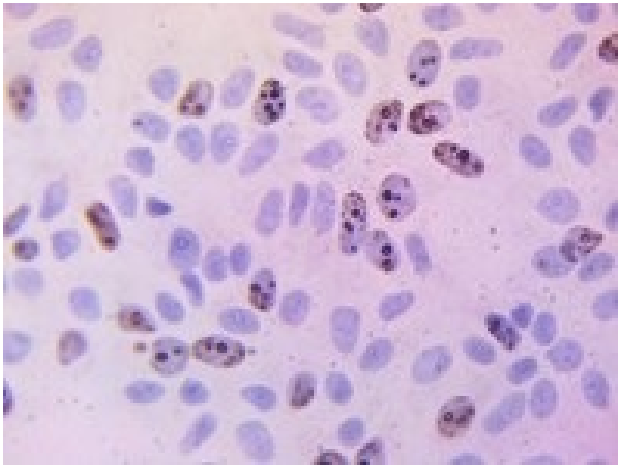
* Hydrogen peroxide is not stable for long periods of time. Be sure the reagent you are using has not expired.

** 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.

8. Sample Images

Example images of formalin-fixed, Formalin fixed MCF7 cells stained using ab125306.





9. Troubleshooting

1. **Poor Positive Staining or No Positive Staining with Little or No Background Staining**

    - Little or no BrdU labeling occurred in the tissue or cells prior to preparing the slides.
    - Detector antibody or Streptavidin-HRP reagent was omitted or used in the wrong order.
    - Use a longer incubation times for Detector Antibody.
    - Use a longer incubation time for substrate (view slide while it is developing).
    - Since excessive counterstaining can compromise positive brown DAB staining, try using shorter hematoxylin counterstain incubation time.
    - DO NOT LET SLIDES DRY OUT; keep wet at all times during the staining procedure.
    - Insufficient blotting between blocking step and detector antibody step. This could dilute out the Detector Antibody component.
    - If tissue is formalin fixed and digestion of the tissue is necessary, the trypsin component may need titering.

- Use fresh xylene solution as solution which has been used many times will contain residual paraffin and may interfere with staining.

2. High Background Staining

- Reduce substrate incubation time.
- Check to make sure the substrate-DAB reagent was prepared correctly (the right ratio of DAB Solution 1 to DAB Solution 2).
- Reduce concentration of the Streptavidin-HRP Component.
- Increase the number and time of washes in between steps.
- Slides incorrectly deparaffinized (use fresh reagents, xylene and ethanol for the de-paraffinization procedure).

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