TROUBLESHOOTING

- Possible causes of negative or poor staining:
  1. BrdU labeling was not adequate.
  2. Staining steps were not performed correctly, or were omitted.
  3. Longer incubation of biotinylated antibody may be required.
  4. Tissue, if fixed in formalin, may need further digestion.
- Possible causes for high background staining:
  1. Tissue may require a longer blocking step.
  2. Inadequate rinsing of slides.
  3. Deparaffinization was not complete.
  4. Over-development of substrate may have occurred.

50 SLIDE KIT CONTAINS (93-3943)		VOl.
Reagent 1A	Trypsin Concentrate	3 ml
Reagent 1B	Trypsin Diluent	12 ml
Reagent 2	Denaturing Solution (Ready-to-use)	6 ml
Reagent 3	Blocking Solution (Ready-to-use)	6 ml
Reagent 4	Biotinylated Ms x BrdU (Ready-to-use)	6 ml
Reagent 5	Streptavidin-peroxidase (Ready-to-use)	6 ml
Reagent 6A	Substrate Buffer Concentrate (20x)	2 ml
Reagent 6B	DAB Concentrate (20x)	2 ml
Reagent 6C	0.6% Hydrogen Peroxide Concentrate (20x)	2 ml
Reagent 7	Hematoxylin (Ready-to-use)	6 ml
Reagent 8	Histomount™ (Ready-to-use)	6 ml
Pos. Control:	Four unstained BrdU control slides	
Reference:	One stained BrdU-positive reference slide	

250 SLIDE KIT CONTAINS (93-3944)		VOl.
Reagent 1A	Trypsin Concentrate	15 ml
Reagent 1B	Trypsin Diluent	60 ml
Reagent 2	Denaturing Solution (Ready-to-use)	30 ml
Reagent 3	Blocking Solution (Ready-to-use)	30 ml
Reagent 4	Biotinylated Ms x BrdU (Ready-to-use)	30 ml
Reagent 5	Streptavidin-peroxidase (Ready-to-use)	30 ml
Reagent 6A	Substrate Buffer Concentrate (20x)	10 ml
Reagent 6B	DAB Concentrate (20x)	10 ml
Reagent 6C	0.6% Hydrogen Peroxide Concentrate (20x)	10 ml
Reagent 7	Hematoxylin (Ready-to-use)	30 ml
Reagent 8	Histomount™ (Ready-to-use)	30 ml
Pos. Control:	Nine unstained BrdU control slides	
Reference:	One stained BrdU-positive reference slide	

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

– Phosphate Buffered Saline (PBS)
– Quenching solution for endogenous peroxidase
– Alcohol and xylene
– Cover slips

CONDITIONS FOR USE

Zymed's BrdU staining kit is designed for research use only and is not intended for therapeutic or diagnostic purposes. Zymed Laboratories, Inc., Zymed sales agents, and distributors will take no responsibility for BrdU kits used in a manner that directly or indirectly violates local regulations or patents. Neither Zymed nor its sales agents can be held responsible for any patent infringement which may occur as the result of improper use of this product.

HANDLING, STORAGE AND SHELF-LIFE

Store kit at 2-8°C. All performance guarantees are void after kit expiration date. Observe necessary health and safety precautions when using this product. Avoid contact with skin and clothes. Wearing of latex or rubber gloves is recommended. There is a potential hazard of explosion due to the reaction of sodium azide, a preservative, with copper metal in plumbing systems. To avoid this, flush the drain thoroughly with water after disposal of reagents.

RELATED PRODUCTS

Zymed supplies a BrdU labeling reagent with recommended procedures (Cat. No. 00-0103).

REFERENCES

1. Ellwart E, Dormer P: Effect of 5-Fluoro-2'-Deoxyuridine (FdUrd) on 5-Bromo-2'-Deoxyuridine (BrdUrd) incorporation into DNA measured with monoclonal BrdUrd antibody and by the BrdUrd/Hoechst Quenching Effect. *Cytometry* 6:513-520, 1985.
2. Gonchoroff NJ et al: S-Phase detection with an antibody to bromodeoxyuridne. *Journal of Immunological Methods* 97-101, 1986.
3. Fukuda K et al: Immunocytochemical detection of S-Phase cells in normal and neoplastic cervical epithelium by anti-BrdU monoclonal antibody. *Analytical and Quantitative Cytology and Histology* Vol. 12 No. 2, pp135-138, April 1990.

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ZYMED® BrdU STAINING KIT

STREPTAVIDIN-BIOTIN SYSTEM

For BrdU Staining

CAT. NO.	GOOD FOR
93-3944	250 Slides

INTRODUCTION

In the past, most cell-proliferation studies have used radioactive thymidine as an incorporated label to give evidence of DNA replication. Recently, a less hazardous technique has been developed using bromodeoxyuridine (BrdU), a thymidine analog, in lieu of a radioactive reagent.(1) BrdU is incorporated into proliferating cells (S-phase) much in the same way as radioactive thymidine, but is then detected by a monoclonal anti-BrdU antibody, and revealed using a highly sensitive streptavidin-biotin staining system. BrdU has proven useful for proliferative studies of normal and neoplastic tissues both *in vivo* and *in vitro*. Zymed's BrdU staining system uses a biotinylated monoclonal anti-BrdU, thus eliminating the need for a species-specific secondary antibody. **As a result, BrdU labeling can be performed in rats and mice without problems of cross-reactivity or spurious staining.** Streptavidin-peroxidase is used as a signal generator for the BrdU system. Diaminobenzidine (DAB) in the presence of hydrogen peroxide is used as a chromogen, staining BrdU-incorporated nuclei dark brown. This kit also contains five BrdU control slides for the convenience of the investigator: one stained reference slide and four unstained positive controls.

SUGGESTED STAINING PROCEDURES:

A. FOR PARAFFIN-EMBEDDED TISSUES

PREPARATION OF SLIDES:

1. Tissues should be labeled with BrdU (Zymed provides BrdU labeling reagent with complete instructions, Cat. No. 00-0103).
2. Fix target tissue in 10% NBF (Neutral Buffered Formalin), or in an alcohol-based fixative (such as alcohol or Methacarn). Process tissues for paraffin embedding.
3. Cut tissue 3-4 microns thick and place on Zymed's HistoGrip™ (Cat. No.00-8050) or poly-l-lysine coated slides. Dry slides in a 60°C oven for 30-60 minutes.
4. Deparaffinize slides in 2 changes of xylene for 5 minutes each. Rehydrate slides in a series of graded alcohol. Slides are now ready for BrdU staining.
5. Formalin-fixed tissues require trypsin digestion.
6. (Optional): If alcohol-fixed tissues are used, tissue sections can be post-dipped in 10% NBF for 30-60 seconds. Rinse well. This may improve morphology.

REAGENT PREPARATION	STAINING PROCEDURES	INCUBATION TIME (Min.)
PEROXIDASE QUENCHING SOLUTION: Add one part 30% H_2O_2 to 9 parts absolute methanol. Mix well.	Submerge slides in Quenching solution. After incubation, rinse with PBS (2 min., 3 times).	10
* **TRYPSIN:** Add 1 drop of reagent 1A to 3 drops of reagent 1B. Mix well.	**FOR FORMALIN FIXED TISSUE ONLY (Not necessary for alcohol fixed tissues).** Add 2 or more drops to each section. Incubate in moist chamber at 37°C. Rinse in distilled water (2 min., 3 times)	3-10
DENATURING SOLUTION: ** Reagent 2 (Ready-to-use)	Apply 2 drops or more to each section. Incubate at room temperature. Rinse with PBS (2 min., 3 times)	20-30
BLOCKING SOLUTION: Reagent 3 (Ready-to-use)	Apply 2 drops or 100 µl to each section. Incubate at room temperature. Drain or blot off the solution. Do not rinse.	10
BIOTINYLATED MOUSE ANTI-BrdU: Reagent 4 (Ready-to-use).	Apply 2 drops or 100 µl to each section. Incubate at room temperature. Rinse with PBS (2 min, 3 times)	30-60
STREPTAVIDIN-PEROXIDASE: Reagent 5 (Ready-to-use).	Apply 2 drops or 100 µl to each section. Incubate at room temperature. Rinse with PBS (2 min, 3 times)	10
DAB MIXTURE: Add 1 drop of reagents 6A, 6B, and 6C to 1 ml distilled water. Mix well. Protect from light and use within one hour.	Apply 2 or more drops of DAB MIXTURE to each section. Incubate. Rinse well with distilled water.	2-5
HEMATOXYLIN: Reagent 7 (Ready-to-use) .	Counterstain the slides with 2 drops or 100 µl of HEMATOXYLIN. Wash slides in tap water. Put slides into PBS until sections turn blue (approx. 30 seconds). Rinse in distilled water.	1-5
HISTOMOUNT™: Reagent 8 (Ready-to-use).	Dehydrate slides in a graded series of alcohol, and clear with xylene. Add 2 drops of Histomount and coverslip.	

* Depending upon the fixative condition, concentration of Trypsin may be varied within a range (dilute 1A:1B from 1:10 to 1:2).

** Corrosive reagent, handle with caution.

B. FOR CULTURED CELLS AND CELL SUSPENSIONS

PREPARATION OF CELLS:

1. Remove labeling medium from cells and wash in several changes of PBS.
2. Fix cells in 70% alcohol or acid-ethanol for 15-30 minutes at 4°C. (Acetone or Methacarn fixatives also can be used.)
3. If necessary, block for endogenous peroxidase activity with 3% hydrogen peroxide in methanol for 10 minutes.
4. Wash in 3 changes of distilled water for 2 minutes each.

REAGENT PREPARATION	STAINING PROCEDURES	INCUBATION TIME (Min.)
DILUTE DENATURING SOLUTION** (Reagent 2) 1:1 with distilled water.	Incubate with Denaturing solution. After incubation, rinse in PBS (2 min, 3 times)	30
BLOCKING SOLUTION: Reagent 3 (Ready-to-use).	Apply sufficient quantity to cover specimen. Incubate at room temperature. Drain or blot off the solution. DO NOT RINSE.	10
BIOTINYLATED MOUSE ANTI-BrdU: Reagent 4 (Ready-to-use).	Add sufficient antibody to cover specimen. Incubate at room temperature. Rinse with PBS (2 min, 3 times).	30-60
STREPTAVIDIN-PEROXIDASE: Reagent 5 (Ready-to-use).	Add sufficient antibody to cover specimen. Incubate at room temperature. Rinse with PBS (2 min, 3 times).	10
DAB MIXTURE: Add 1 drop of reagents 6A, 6B, and 6C to 1 ml distilled water. Mix well. Protect from light and use within one hour.	Add sufficient quantity of the DAB MIXTURE to cover specimen. Incubate. Rinse well with distilled water.	2-5
HEMATOXYLIN: Reagent 7 (Ready-to-use).	Counterstain with sufficient quantity of HEMATOXYLIN to cover specimen. Wash slides in tap water. Put slides into PBS until sections turn blue (approx. 30 seconds). Rinse in distilled water.	1-2
HISTOMOUNT™: Reagent 8 (Ready-to-use).	Dehydrate slides in a graded series of alcohol, and clear with xylene. Add 2 drops of Histomount and coverslip.	

** Corrosive reagent, handle with caution.