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 Mx x BrdU (Ready-to-use) 6 ml
Reagent 5 Streptavidin-peroxidase (Ready-to-use) 6 ml
Reagent 6A Substrate Buffer Concentrate (2x) 2 ml
Reagent 6B DAB Concentrate (2x) 2 ml
Reagent 6C 0.6% Hydrogen Peroxide Concentrate (2x) 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 Mx x BrdU (Ready-to-use) 30 ml
Reagent 5 Streptavidin-peroxidase (Ready-to-use) 30 ml
Reagent 6A Substrate Buffer Concentrate (2x) 10 ml
Reagent 6B DAB Concentrate (2x) 10 ml
Reagent 6C 0.6% Hydrogen Peroxide Concentrate (2x) 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

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

REFERENCES

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.

ZYMED® BrdU Staining Kit

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. 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.

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4/6/2005
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**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.

<table>
<thead>
<tr>
<th>Reagent Preparation</th>
<th>Staining Procedures</th>
<th>Incubation Time (Min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEROXIDASE QUenchING SOLUTION:</td>
<td>Submerge slides in Quenching solution. After incubation, rinse with PBS (2 min., 3 times).</td>
<td>10</td>
</tr>
<tr>
<td>* TRYPsin:</td>
<td>Add 1 drop of reagent 1A to 3 drops of reagent 1B. Mix well.</td>
<td>3-10</td>
</tr>
<tr>
<td>DENATURING SOLUTION:</td>
<td>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)</td>
<td></td>
</tr>
<tr>
<td>** Reagent 2 (Ready-to-use)</td>
<td>Apply 2 drops or more to each section. Incubate at room temperature. Rinse with PBS (2 min., 3 times).</td>
<td>20-30</td>
</tr>
<tr>
<td>BLOCKING SOLUTION:</td>
<td>Reagent 3 (Ready-to-use)</td>
<td>10</td>
</tr>
<tr>
<td>Biotinylated Mouse Anti-BrdU:</td>
<td>Reagent 4 (Ready-to-use).</td>
<td>30-60</td>
</tr>
<tr>
<td>Streptavidin-Peroxidase:</td>
<td>Reagent 5 (Ready-to-use).</td>
<td>10</td>
</tr>
<tr>
<td>DAB MIXTURE:</td>
<td>Add 1 drop of reagents 6A, 6B, and 6C to 1 ml distilled water. Mix well. Protect from light and use within one hour.</td>
<td>2-5</td>
</tr>
<tr>
<td>HEMATOXYLIN:</td>
<td>Reagent 7 (Ready-to-use).</td>
<td>1-5</td>
</tr>
<tr>
<td>HISTOMOUNT™:</td>
<td>Reagent 8 (Ready-to-use).</td>
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* Depending upon the fixative condition, concentration of Trypsin may be varied within a range (dilute 1A:1B from 1:10 to 1:2).

### 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.

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<tr>
<td>Dilute Denaturing Solution**</td>
<td>Incubate with Denaturing solution. After incubation, rinse in PBS (2 min., 3 times).</td>
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<td>Blocking Solution:</td>
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**Corrosive reagent, handle with caution.