00-0103 15 ml
BrdU Labeling Reagent
Lot No.

## BrdU LABELING REAGENT

The BrdU labeling reagent is supplied as a concentrated aqueous solution of 5-bromo-2'-deoxyuridine and 5-fluoro-2'-deoxyuridine (10:1).

Storage: Store at 2-8ºC

### Procedures for Labeling and Preparation of Slides for Immunohisto/ cytochemical Staining:

### Labeling with BrdU In Vivo

1. Inject the animal with labeling reagent. As a general rule, 1 ml concentrated reagent per 100 g body weight is a suitable amount. Intraperitoneal injections are recommended for mice.
   **Note: For larger animals use 10 ml per 1 kg of body weight.**
2. Wait 2 hours and sacrifice the animal. Remove the organs you wish to study.
3. Process the tissue as necessary (frozen sections or paraffin embedding).
4. Cut sections for immunohistochemistry.

### Labeling Culture Cells or Cell Suspensions in Flask

1. Use a standard protocol to grow up cell cultures in vitro.
2. Dilute the labeling reagent 1:100 (from concentrate) with complete tissue culture medium. Filter sterilize and warm to 37°C before use.
3. Remove the cell culture medium, and replace it with the diluted labeling solution.
4. Incubate cells at 37°C for 60 minutes to overnight. The optimum incubation time will depend upon cell type and the goal of the experiment.
5. Prepare slides carrying cell monolayers by using one of the following procedures:
   A. Cytospin preparation:
   Cytocentrifuge 100 µL of labeled cells at a concentration of 1-2 x $10^6$ cells per ml directly onto cleaned slides and air dry.
   B. Cell-smear preparation:
   Place one small drop of labeled cell suspension onto the end of a glass slide and spread into a thin film. This is easily accomplished using the edge of a second slide. Air dry at room temperature.
6. Fix cells by placing slides in 70-80% alcohol or acid-ethanol for 20-30 min.
7. Wash with PBS (3 times, 2 min. each)
8. Proceed with immunocytochemical staining techniques.

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288
E-mail: techsupport@invitrogen.com

PI000103 (Rev 10/09) DCC-09-1535



00-0103 15 ml
BrdU Labeling Reagent
Lot No.

BrdU LABELING REAGENT

The BrdU labeling reagent is supplied as a concentrated aqueous solution of 5-bromo-2'-deoxyuridine and 5-fluoro-2'-deoxyuridine (10:1).

Storage: Store at 2-8ºC

Procedures for Labeling and Preparation of Slides for Immunohisto/ cytochemical Staining:

Labeling with BrdU In Vivo

1. Inject the animal with labeling reagent. As a general rule, 1 ml concentrated reagent per 100 g body weight is a suitable amount. Intraperitoneal injections are recommended for mice.
   Note: For larger animals use 10 ml per 1 kg of body weight.
2. Wait 2 hours and sacrifice the animal. Remove the organs you wish to study.
3. Process the tissue as necessary (frozen sections or paraffin embedding).
4. Cut sections for immunohistochemistry.

Labeling Culture Cells or Cell Suspensions in Flask

1. Use a standard protocol to grow up cell cultures in vitro.
2. Dilute the labeling reagent 1:100 (from concentrate) with complete tissue culture medium. Filter sterilize and warm to 37°C before use.
3. Remove the cell culture medium, and replace it with the diluted labeling solution.
4. Incubate cells at 37°C for 60 minutes to overnight. The optimum incubation time will depend upon cell type and the goal of the experiment.
5. Prepare slides carrying cell monolayers by using one of the following procedures:
   A. Cytospin preparation:
   Cytocentrifuge 100 µL of labeled cells at a concentration of 1-2 x $10^6$ cells per ml directly onto cleaned slides and air dry.
   B. Cell-smear preparation:
   Place one small drop of labeled cell suspension onto the end of a glass slide and spread into a thin film. This is easily accomplished using the edge of a second slide. Air dry at room temperature.
6. Fix cells by placing slides in 70-80% alcohol or acid-ethanol for 20-30 min.
7. Wash with PBS (3 times, 2 min. each)
8. Proceed with immunocytochemical staining techniques.

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288
E-mail: techsupport@invitrogen.com

PI000103 (Rev 10/09) DCC-09-1535

Important Licensing Information - These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, www.invitrogen.com). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.

## Labeling Culture Cells on Chamber Slides

1. Culture cells overnight on 4-well chamber slides.
2. Prepare labeling solution by diluting labeling reagent 1:100 (from concentrate) with complete tissue culture medium. Filter, sterilize and warm to 37°C before use.
3. Remove medium from chamber slides and replace with labeling solution.
4. Incubate cells at 37°C in a $CO_2$ incubator for 2 hours to overnight, depending upon the goal of the experiment.
5. Remove labeling medium from cells and wash gently with PBS, 2 times.
6. Fix cells with 70-80% alcohol or acid alcohol for 20-30 min.
7. Wash with PBS (3 times, 2 min. each)
8. Proceed with immunocytochemical staining techniques.

## Labeling of Tissue Slices

1. Dilute the labeling reagent from 1:50 to 1:100 (from concentrate), using complete tissue culture medium. Sterilize by running through a 0.22 micron filter. Warm to 37°C before use. Prepare only the volume of labeling solution required for each experiment, while making sure there is enough labeling reagent to thoroughly immerse the specimen.
2. Cut the tissue sample with a scalpel or sharp blade, making slices about 1mm thick and 2 $mm^2$ in area. It is recommended that the cutting be performed in prewarmed tissue culture medium. This will help maintain tissue viability.
3. Transfer tissue slices to a 15 ml cell culture tube filled with 10 ml of labeling medium, and prewarmed to 37°C. Incubate at 37°C in a $CO_2$ incubator for the required labeling time. As an option, add 100 µL of 30% (v/v) hydrogen peroxide before sealing the tube, and incubate at 37°C in a conventional incubator. This time will vary, depending upon the type of tissue slices used, and the goal of the experiment (usually 2 to 4 hours). Discard any unused labeling medium.
4. Wash tissue slices in PBS at 37°C (3 times, 5 minutes each).
5. Fix the tissue using a frozen section protocol or embed in paraffin as necessary.

For research use only. CAUTION: Not intended for human or animal therapeutic or diagnostic use.

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288
E-mail: techsupport@invitrogen.com

PI000103 (Rev 10/09 ) DCC-09-1535



## Labeling Culture Cells on Chamber Slides

1. Culture cells overnight on 4-well chamber slides.
2. Prepare labeling solution by diluting labeling reagent 1:100 (from concentrate) with complete tissue culture medium. Filter, sterilize and warm to 37°C before use.
3. Remove medium from chamber slides and replace with labeling solution.
4. Incubate cells at 37°C in a $CO_2$ incubator for 2 hours to overnight, depending upon the goal of the experiment.
5. Remove labeling medium from cells and wash gently with PBS, 2 times.
6. Fix cells with 70-80% alcohol or acid alcohol for 20-30 min.
7. Wash with PBS (3 times, 2 min. each)
8. Proceed with immunocytochemical staining techniques.

## Labeling of Tissue Slices

1. Dilute the labeling reagent from 1:50 to 1:100 (from concentrate), using complete tissue culture medium. Sterilize by running through a 0.22 micron filter. Warm to 37°C before use. Prepare only the volume of labeling solution required for each experiment, while making sure there is enough labeling reagent to thoroughly immerse the specimen.
2. Cut the tissue sample with a scalpel or sharp blade, making slices about 1mm thick and 2 $mm^2$ in area. It is recommended that the cutting be performed in prewarmed tissue culture medium. This will help maintain tissue viability.
3. Transfer tissue slices to a 15 ml cell culture tube filled with 10 ml of labeling medium, and prewarmed to 37°C. Incubate at 37°C in a $CO_2$ incubator for the required labeling time. As an option, add 100 µL of 30% (v/v) hydrogen peroxide before sealing the tube, and incubate at 37°C in a conventional incubator. This time will vary, depending upon the type of tissue slices used, and the goal of the experiment (usually 2 to 4 hours). Discard any unused labeling medium.
4. Wash tissue slices in PBS at 37°C (3 times, 5 minutes each).
5. Fix the tissue using a frozen section protocol or embed in paraffin as necessary.

For research use only. CAUTION: Not intended for human or animal therapeutic or diagnostic use.

www.invitrogen.com
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288
E-mail: techsupport@invitrogen.com

PI000103 (Rev 10/09) DCC-09-1535

**Important Licensing Information** - These products may be covered by one or more Limited Use Label Licenses (see the Invitrogen Catalog or our website, www.invitrogen.com). By use of these products you accept the terms and conditions of all applicable Limited Use Label Licenses. Unless otherwise indicated, these products are for research use only and are not intended for human or animal diagnostic, therapeutic or commercial use.