ab126556 BrdU Cell Proliferation ELISA Kit (Colorimetric)

For the detection of BrdU incorporation into newly synthesized DNA of actively proliferating cells. For research use only – not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab126556
(use abcam.cn/ab126556 for China, or abcam.co.jp/ab126556 for Japan)

Storage and Stability:
Store kit at +4°C immediately upon receipt except for the Prediluted Anti-BrdU Detector Antibody and Peroxidase Goat anti-mouse IgG (2000X) these components must be stored at -20°C. Refer to list of materials supplied for storage conditions of individual components before and after preparation. Prediluted anti-BrdU Detector Antibody and Peroxidase Goat anti-mouse IgG (2,000X) should be aliquoted before being stored at -20°C to reduce freeze thaw cycles.

Materials Supplied:

Item	Amount	Storage Condition (Before Preparation)
500X BrdU Reagent	15 µL	+2 - 8°C
Fixing Solution	2 x 20 mL	+2 - 8°C
Prediluted anti-BrdU Detector Antibody	20 mL	- 20°C
Stop Solution	25 mL	+2 - 8°C
Peroxidase Goat anti-mouse IgG (2,000X)	15 µL	- 20°C
Conjugate Diluent	25 mL	+2 - 8°C
TMB Peroxidase Substrate	25 mL	+2 - 8°C
Plate Wash Concentrate (50X)	90 mL	+2 - 8°C

Δ Note: Prediluted anti-BrdU Detector Antibody and Peroxidase Goat anti-mouse IgG (2,000X) should be aliquoted before being stored at -20°C to reduce freeze thaw cycles.

Materials Required, Not Supplied:

- 2-20 µL, 20-200 µL and 200-1,000 µL precision pipettors with disposable tips.
- Wash bottle or multichannel dispenser for washing.
- 2X Test reagent (optional).
- The test reagent can be a cell proliferation enhancer or alternatively, can induce growth inhibition or arrest. The test reagent is diluted to twice the desired final concentration (2X) in the cell media used.
- 2,000 mL graduated cylinder.
- PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4-$7H_2O$, 1.4 mM KH_2PO_4).
- Deionized or distilled H_2O.
- Spectrophotometer capable of measuring absorbance in 96 well plates using dual wavelength of 450-540 or 450-595 nm or a single read at 450 nm.
- Tissue culture microtiter plates (96 well culture dish).
- Sterile reagent troughs.
- Micro syringe filter (0.2 µm).
- Syringe.

Reagent Preparation:

Equilibrate all reagents to room temperature (18-25°C) for 4 hours prior to use.
Prepare only as much reagent as is needed on the day of the experiment.

1. **1X BrdU Reagent**
   Dilute the 500X concentrated stock 500-fold by adding 6 µL of BrdU stock to 3 mL of cell media.
2. **1X Plate Wash Buffer**
   Dilute the 50X Plate Wash Concentrate 50-fold by adding 40 mL to 1,960 mL of distilled water.
3. **Peroxidase Goat Anti-Mouse IgG Conjugate**
   Dilute the Peroxidase Goat Anti-Mouse IgG Conjugate 2,000-fold by adding 6 µL to 12 mL of the Conjugate Diluent provided. Once diluted, this solution should be filtered using a 0.22 µm syringe filter. This lowers the assay background and improves precision.
4. **Fixing solution**
   The reagent is provided ready to use. The Fixative/Denaturing Solution may contain slight precipitation and its color may vary between clear to light yellow
5. **Prediluted anti-BrdU Detector Antibody**
   The reagent is provided ready to use.

Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all controls and samples in duplicate.
- Do not expose reagents to excessive light. Do not mix reagents from different kits. The buffers and reagents used in this kit contain anti-microbial and anti-fungal reagents. Care should be taken to prevent direct contact with these products.
- Two types of controls are recommended to insure validity of the experiment.
  Blank: Add only tissue culture media (no cells).
  Background: Cells are present in the wells but do not add the BrdU Reagent.

1. **Cell Plating**
   Seed cells using a sterile 96-well tissue culture plate, cells are plated at 2 x 10^5 cells/mL in 100 µL/well of appropriate cell culture media. Some of the wells on the plate should be set aside for several controls. These should include wells that do not receive cells (media alone), and wells which contain cells but will not receive the BrdU reagent (assay background).

2. **Addition of test Reagent**
   The test reagent can be a cell proliferation enhancer or alternatively, can induce growth inhibition or arrest. Dilute the test reagent to twice the desired final concentration (2X) in the cell media. Pipette 100 µL/well on top of the cell wells. The test reagent should be titered in the assay to determine optimum concentration for inducing cell proliferation or growth arrest. The length of time for test reagent incubation should also be determined for your system (time course study). BrdU addition (see step 3 below) will occur 2-24 hours prior to the end of the test reagent incubation.

3. **Addition of BrdU**
   Pipette 20 µL of the diluted 1X BrdU label to the appropriate wells. Incubate the assay 2-24 hours. BrdU will be incorporated into proliferating cells and should be added at least 2 hours prior to the end of the test reagent incubation period. Better sensitivity and signal to noise ratios are obtained when longer BrdU labelling times are used.

Δ Note: A series of wells should be set aside that do NOT receive the BrdU label (negative BrdU control for determining assay background).

4. **Fix and Denature Step and Storage od Fixed Plates**
For detection of the BrdU label by the anti-BrdU monoclonal antibody, it is necessary to fix the cells and denature the DNA using Fixing Solution. There is no need to spin the cells prior to addition of the fixing solution. However, if suspension cells are being used, better precision is obtained if the cell plates are spun in a centrifuge prior to the fix/denature step.
Δ Note: Fixed plates can be stored for up to 1 month at +2-8°C if stored in a heat sealed or zip-lock bag. If storing your plates for future use, make sure the plates are blotted well and are very dry (NO Fixing Solution should be left in the wells).

Adherent Cells (No-Spin Procedure)
Aspirate the media from the cell wells (this can be done mechanically or plate can be inverted over appropriate reservoir and blotted on absorbent paper towels). Add 200 µL/well Fixing Solution and incubate at room temperature for 30 minutes. Aspirate the Fixing Solution and blot the plate dry. The assay can be run immediately or plates may be stored for future use (see note above).

Suspension Cells (Spin Fix/Denature Procedure)
Spin the plates in the centrifuge (using appropriate centrifuge microtiter plate holders) for 5 minutes at 1,000 rpm. Aspirate the media and add 200 µL/well Fixing Solution. Incubate for 30 minutes at room temperature. Aspirate the Fixing Solution and blot the plates dry. The cells are now adhered to the plate. The assay can be run immediately or plates may be stored for future use (see note above).

5. **Wash step**
Wash the plate three times with 1X Wash Buffer prior to adding Detector Antibody. Aspirate the wash solution after the final wash and blot dry on paper towels. A microtiter plate washer may be used for all wash steps OR a squirt bottle for manual plate washing may also be used. In either case, the wells should be filled completely with wash buffer

6. **Addition on Detector antibody**
Add 100 µL/well anti-BrdU monoclonal Detector Antibody and incubate for 1 hour at room temperature.

7. **Wash step**
Wash as in Step 5 above.

8. **Preparation and Addition of the peroxidase Goat Anti-Mouse igG Conjugate**
Pipette 100 µL/well 1X Peroxidase Goat Anti-Mouse IgG Conjugate and incubate for 30 minutes at room temperature.

9. **Wash Step and Final Water Wash**
Wash as in Step 5 above. Perform a final water wash by flooding the entire plate with distilled water. Pat dry on absorbent paper towels.

10. **Addition of TMB Peroxidase Substrate**
Pipette 100 µL/well TMB Peroxidase substrate and incubate for 30 minutes at room temperature in the dark. Positive wells will be visible by a blue color, the intensity of which is proportional to the amount of BrdU incorporation in the proliferating cells.

11. **Addition of Stop Solution and Reading of the Plate**
Stop the reaction by pipetting 100 µL of Stop Solution provided to every well. The color of positive wells will change from blue to bright yellow. Read the plate using a spectrophotometric microtiter plate reader set at a dual wavelength of 450/550 nm (alternatively, 450/540 nm or 450/595 nm may be used or a single read at 450 nm).
Δ Note: Dual wavelength allows to read the plate at a wavelength specific for the reaction product and at a second wavelength that serves as reference and is out of the range of absorbance for the reaction product. The second wavelength is subtracted from the first, effectively subtracting out the background noise that is usually not wavelength specific.

Assay Sensitivity:

A sensitivity study was performed using the Jurkat (non-adherent) and RH7777 and MCF7 (adherent) cells. Various concentrations of the cells were plated and cultured for 24 hours. The cells were incubated with BrdU Label for 24 hours and incorporated BrdU was detected with the BrdU cell proliferation ELISA Kit. There was a direct relationship between the signal and number of proliferating cells at all cell concentrations. The sensitivity of this assay was determined to be 40 cells/well using the mean signal of zero plus two standard deviations; that is, the smallest number of cells that may be distinguished from zero with 95% confidence. Using a two-hour BrdU labeling, 100 cells/well was also significantly higher than the blank control.

Technical Support

Copyright © 2026 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)