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# ab126556 – BrdU Cell Proliferation ELISA Kit (colorimetric)

For the detection of BrdU incorporation into newly synthesized DNA of actively proliferating cells.

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

## Table of Contents

1. Overview	1
2. Protocol Summary	3
3. Precautions	4
4. Storage and Stability	4
5. Limitations	5
6. Materials Supplied	5
7. Materials Required, Not Supplied	6
8. Technical Hints	7
9. Reagent Preparation	8
10. Assay Procedure	9
– Equilibrate all materials and prepared reagents to room temperature prior to use.	9
11. Assay Sensitivity	13
12. Troubleshooting	14
13. Notes	16
<b>Technical Support</b>	<b>18</b>

# 1. Overview

Abcam's BrdU Cell Proliferation ELISA Kit (colorimetric) in vitro ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for accurate detection of DNA synthesis and cell proliferation.

Abcam's BrdU Cell Proliferation ELISA Kit involves incorporation of BrdU into cells cultured in microtiter plates using the cell layer as the solid phase. During the final 2 to 24 hours of culture BrdU is added to wells of the microtiter plate. BrdU will be incorporated into the DNA of dividing cells. To enable antibody binding to the incorporated BrdU cells must be fixed, permeabilized and the DNA denatured. This is all done in one step by treatment with Fixing Solution. Detector anti-BrdU monoclonal antibody is pipetted into the wells and allowed to incubate for one hour, during which time it binds to any incorporated BrdU. Unbound antibody is washed away and horseradish peroxidase-conjugated goat anti-mouse antibody is added, which binds to the Detector Antibody.

The horseradish peroxidase catalyzes the conversion of the chromogenic substrate tetra-methylbenzidine (TMB) from a colorless solution to a blue solution (or yellow after the addition of stopping reagent), the intensity of which is proportional to the amount of incorporated BrdU in the cells. The colored reaction product is detected using a spectrophotometer.

Evaluation of cell cycle progression is essential for investigations in many scientific fields. Measurement of [3H] thymidine incorporation as cells enter S phase has long been the traditional method for the detection of cell proliferation. Subsequent quantification of [3H] 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 [3H] thymidine uptake has been demonstrated by numerous investigators. In these methods bromodeoxyuridine (BrdU), a thymidine analog replaces [3H] 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.

Abcam's BrdU Cell Proliferation ELISA Kit assay is sensitive, rapid, easy to perform and applicable to high sample throughput. In addition to evaluation of cell proliferation, information such as cell number, morphology and analysis of cellular antigens can be obtained from a single culture.

## 2. Protocol Summary

Prepare all reagents, samples, and standards as instructed, seed cells and add test sample if required.



Add BrdU. Incubate for 2-24hours. Proliferating cells will incorporate BrdU into their DNA.



Add Fixing Solution and incubate to fix the cells and denature the DNA.



Aspirate and wash each well. Add Primary detector antibody to each well used. Incubate at room temperature



Aspirate and wash each well. Add prepared HRP conjugate antibody to each well. Incubate at room temperature.



Aspirate and wash each well. Add the TMB Solution to each well. After color develops and then add the Stop Solution. Immediately begin recording the color development

### 3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

### 4. 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. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

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.

## 5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

## 6. Materials Supplied

Item	Amount	Storage Condition (Before Preparation )
500X BrdU Reagent	15 $\mu$ L	+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 $\mu$ L	- 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

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- 2-20  $\mu\text{L}$ , 20-200  $\mu\text{L}$  and 200-1,000  $\mu\text{L}$  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  $\text{Na}_2\text{HPO}_4\cdot 7\text{H}_2\text{O}$ , 1.4 mM  $\text{KH}_2\text{PO}_4$ ).
- Deionized or distilled  $\text{H}_2\text{O}$ .
- 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  $\mu\text{m}$ ).
- Syringe.



## 8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.
- Complete removal of all solutions and buffers during wash steps is necessary to minimize background.

## 9. 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.

### 9.1 1X BrdU Reagent

Dilute the 500X concentrated stock 500-fold by adding 6 µL of BrdU stock to 3 mL of cell media.

### 9.2 1X Plate Wash Buffer

Dilute the 50X Plate Wash Concentrate 50-fold by adding 40 mL to 1,960 mL of distilled water.

### 9.3 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.

### 9.4 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

### 9.5 Prediluted anti-BrdU Detector Antibody

The reagent is provided ready to use.

## 10. 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.
  - i. Blank: Add only tissue culture media (no cells).
  - ii. Background: Cells are present in the wells but do not add the BrdU Reagent.

### 10.1 Cell Plating

Seed cells using a sterile 96-well tissue culture plate, cells are plated at  $2 \times 10^5$  cells/mL in 100  $\mu$ L/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).

### 10.2 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  $\mu$ L/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 10.3 below) will occur 2-24 hours prior to the end of the test reagent incubation.

### 10.3 Addition of BrdU

Pipette 20  $\mu\text{L}$  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).

### 10.4 Fix and Denature Step and Storage of 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 and Suspension 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  $\mu\text{L}$ /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  $\mu\text{L}$ /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).

## 10.5 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

## 10.6 Addition on Detector antibody

Add 100  $\mu$ L/well anti-BrdU monoclonal Detector Antibody and incubate for 1 hour at room temperature.

## 10.7 Wash step

Wash as in Step 10.5 above.

## 10.8 Preparation and Addition of the peroxidase Goat Anti-Mouse igG Conjugate

Pipette 100  $\mu$ L/well 1X Peroxidase Goat Anti-Mouse IgG Conjugate and incubate for 30 minutes at room temperature.

## 10.9 Wash Step and Final Water Wash

Wash as in Step 10.5 above. Perform a final water wash by flooding the entire plate with distilled water. Pat dry on absorbent paper towels.

## 10.10 Addition of TMB Peroxidase Substrate

Pipette 100  $\mu$ L/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.

## 10.11 Addition of Stop Solution and Reading of the Plate

Stop the reaction by pipetting 100  $\mu$ L 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).

## 11. 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.

## 12. Troubleshooting

<b>Problem</b>	<b>Reason</b>	<b>Solution</b>
<b>Low Precision</b>	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing
<b>Precipitate in Diluent</b>	Precipitation and/or coagulation of components within the Diluent.	Precipitate can be removed by gently warming the Diluent to 37°C.



<b>Problem</b>	<b>Cause</b>	<b>Solution</b>
<b>Low Signal</b>	Incubation times too brief	Ensure sufficient incubation times; increase to 2 or 3 hour standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
<b>Large CV</b>	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions.
	Contaminated wash buffer	Prepare fresh wash buffer
<b>Low sensitivity</b>	Improper storage of the ELISA kit	Store your reconstituted standards at -80°C, all other assay components 4°C. Keep TMB substrate solution protected from light.

# 13. Notes



## Technical Support

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### **Austria**

wissenschaftlicherdienst@abcam.com | 019-288-259

### **France**

supportscientifique@abcam.com | 01.46.94.62.96

### **Germany**

wissenschaftlicherdienst@abcam.com | 030-896-779-154

### **Spain**

soportecientifico@abcam.com | 91-114-65-60

### **Switzerland**

technical@abcam.com

Deutsch: 043-501-64-24 | Français: 061-500-05-30

### **UK, EU and ROW**

technical@abcam.com | +44(0)1223-696000

### **Canada**

ca.technical@abcam.com | 877-749-8807

### **US and Latin America**

us.technical@abcam.com | 888-772-2226

### **Asia Pacific**

hk.technical@abcam.com | (852) 2603-6823

### **China**

cn.technical@abcam.com | +86-21-5110-5938 | 400-628-6880

### **Japan**

technical@abcam.co.jp | +81-(0)3-6231-0940

### **Singapore**

sg.technical@abcam.com | 800 188-5244

### **Australia**

au.technical@abcam.com | +61-(0)3-8652-1450

### **New Zealand**

nz.technical@abcam.com | +64-(0)9-909-7829