BrdU Cell Proliferation In-cell ELISA Kit

BrdU Cell Proliferation In-cell ELISA Kit is an Enzyme Immunoassay kit for the quantification of BrdU incorporated into cells in cell culture supernatants.

Catalog number: ARG82214

Package: 96 assays

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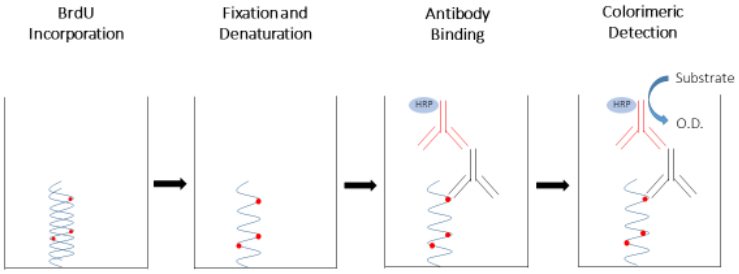
INTRODUCTION

Bromodeoxyuridine (5-bromo-2'-deoxyuridine, BrdU, BUdR, BrdUrd, broxuridine) is a synthetic nucleoside analogue with a chemical structure similar to thymidine. BrdU is commonly used to study cell proliferation in living tissues and has been studied as a radiosensitizer and diagnostic tool in people with cancer. [Provided by Wikipedia: BrdU]

PRINCIPLE OF THE ASSAY

This assay employs the indirect enzyme immunoassay technique. A highly specific antibody for BrdU added into the pre-treated cell culture plate. After incubation and wash step. Then add HRP Conjugate into the plate. After incubation, the wells are washed with diluted Wash Buffer to remove unbound material. TMB substrate is added to the wells and color develops. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450 nm. The magnitude of the absorbance for the developed color is proportional to the quantity of BrdU incorporated into cells and can be directly correlated to cell proliferation.



MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store the 1000X BrdU Solution and Anti-BrdU Antibody at -20°C. Store all other components at 4°C. Use the kit before expiration date.

Component	Quantity	Storage information
1000X BrdU Solution (10 mM)	30 µL	-20°C
1000X Anti-BrdU Antibody	10 µL	-20°C
Fix / Denaturation Solution	20 mL (ready to use)	4°C
1000X HRP Conjugate	20 µL	4°C
Diluent Buffer	50 mL (ready to use)	4°C
10X Wash Buffer	100 mL	4°C
TMB substrate (amber bottle)	12 mL (ready to use)	4°C (protect from light)
STOP solution	12 mL (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Deionized or distilled water
- 1X PBS
- Cell culture medium
- 96-well cell culture plate
- Pipettes and pipette tips
- Microplate washer (recommended)

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, store the 1000X BrdU Solution and Anti-BrdU Antibody at -20°C. Store all other components at 4°C.
- Briefly spin down the all vials before use.
- If crystals are observed in the 10X Wash Buffer, warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

Cell culture:

1. Prepare a cell suspension containing **0.1-1 x 10^6 cells/mL** in cell culture medium.
2. Add **100 µL** per well to a 96-well cell culture plate and incubate overnight at **37°C** and **5% CO_2** in a humidified incubator.
3. Add compound to be tested and a negative control (without compound). Culture the cells for **24-96** hours at **37°C** and **5% CO_2** in a humidified incubator.
4. Add **10 µL** of **10X BrdU solution** to wells and incubate for **1-6 hours** at **37°C** and **5% CO_2** in a humidified incubator.

   Note: optimal time of incubation with BrdU will vary with cell type.
5. Carefully and slow aspirate wells with **100 µL** of **1X PBS**; repeat this wash step 2 more times.
6. After the final aspiration, add **100 µL** of **Fix / Denature Solution** and incubate **30 minutes** at **37°C**.
7. Wash wells 3 times as described in step 5.
8. The cell culture sample (cell culture plate) should be assayed immediately.

REAGENT PREPARATION

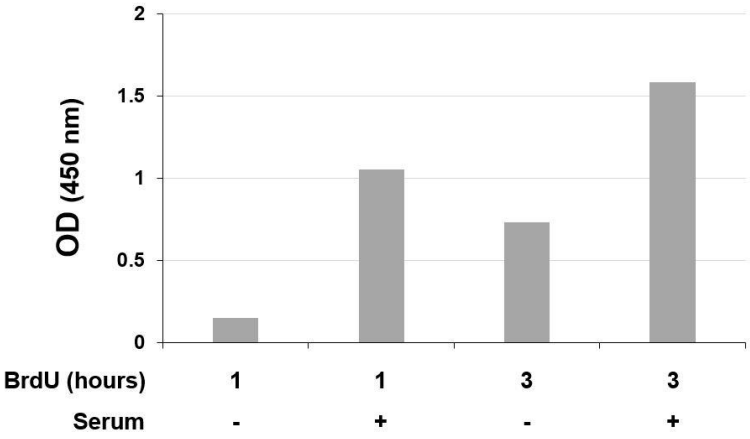
- **1X Wash Buffer:** Dilute 10X Wash Buffer into deionized water to yield 1X Wash Buffer. (E.g., add 50 mL of 10X Wash Buffer into 450 mL of distilled water to a final volume of 500 mL)
- **10X BrdU Solution:** Dilute 1000X stock BrdU solution into cell culture medium to yield 10X BrdU Solution. (E.g., add 1 µL 1000X stock BrdU into 99 µL cell culture medium to a final volume of 100 µL)
- **1X Anti-BrdU Antibody:** Dilute 1000X Anti-BrdU Antibody into Diluent Buffer to yield 1X Anti-BrdU Antibody. (E.g., add 1 µL 1000X Anti-BrdU Antibody into 999 µL Diluent Buffer to a final volume of 1 mL)
- **1X HRP Conjugate:** Dilute 1000X HRP Conjugate into Diluent Buffer to yield 1X HRP Conjugate. (E.g., add 1 µL 1000X HRP Conjugate into 999 µL Diluent Buffer to a final volume of 1 mL)

ASSAY PROCEDURE

1. Add **100 µL** of **Diluent Buffer** into the cell culture plate. (See **SAMPLE COLLECTION & STORAGE INFORMATION** section)
2. Incubate at **room temperature (RT, 20-25°C)** for **1 hour.**
3. Wash wells 3 times with **100 µL** of **1X PBS.**
4. Add **100 µL** of **Anti-BrdU Antibody** into each well.
5. Incubate at **RT** for **60 minutes** on a microplate shaker.
6. Aspirate each well and wash, repeating the process 2 times for a total **3 washes.** Wash by filling each well with **1× Wash Buffer (250 µL)** using a squirt bottle, manifold dispenser, or autowasher. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
7. Add **100 µL** of **HRP Conjugate** to each well.
8. Incubate at **RT** for **60 minutes** on a microplate shaker.
9. Aspirate each well and **wash as step 6.**
10. Add **100 µL** of **TMB Substrate** to each well, including the blank wells. Incubate in the dark at **RT** for **2-30 minutes** on a microplate shaker.
    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
11. Immediately Add **100 µL** of **Stop Solution** to each well, including the blank wells. The color of the solution should change from blue to yellow.
12. Read the OD with a microplate reader at **450 nm** immediately. (Color will fade over time)

EXAMPLE OF RESULT

The following figures demonstrate typical results with the BrdU Cell Proliferation In-cell ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



Serum Stimulation of Proliferation in HEK 293 Cells

HEK 293 Cells were plated overnight at 37°C. Cells were then incubated in the presence or absence of 10% FBS for 24 hours, treated with 10 µM BrdU for 1 or 3 hours, and processed for BrdU incorporation according to the assay protocol.