ELISA PRODUCT INFORMATION & MANUAL

BrdU Cell Proliferation Assay Kit (Colorimetric) (Colorimetric)

NBP2-54888

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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NBP2-54888 – BrdU Cell Proliferation Assay Kit (Colorimetric)

For the detection of incorporated BrdU using a mouse anti-BrdU antibody.
For research use only - not intended for diagnostic use.

Storage and Stability

Store kit at -20°C protected from light. Briefly centrifuge small vials at low speed prior to opening. Read entire protocol before performing the experiment. Prepare following reagents as needed just before use. We do not recommend storing the diluted solutions.

Materials Supplied

Item	200 Tests	100 Tests	Storage Condition
10X Wash buffer	40 ml	200 ml	-20°C
2000X Anti-Mouse HRP-linked Antibody	10 µl	50 µl	-20°C
Antibody Diluent	50 ml	250 ml	-20°C
1000X BrdU	20 µl	100 µl	-20°C
300X BrdU Detection Antibody	70 µl	350 µl	-20°C
Fixing/Denaturing Solution	20 ml	100 ml	-20°C
Stop Solution	20 ml	100 ml	-20°C
TMB Substrate	20 ml	100 ml	-20°C

Materials Required, Not Supplied

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

- Multi-well spectrophotometer
- 96-well clear plate with flat bottom (tissue cell culture treated)

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

BrdU: Prepare 10X BrdU solution by diluting BrdU 1:100 with the cell culture medium.

300X BrdU Detection Antibody: Prepare 1X solution by diluting BrdU Detection Antibody 1:300 with Antibody Diluent.

2000X Anti-mouse HRP-linked Antibody: Prepare 1X solution by diluting the Anti-mouse HRP-linked antibody 1:2000 with Antibody Diluent.

10X Wash Buffer: Prepare 1X solution by diluting with dH2O.

BrdU Cell Proliferation Assay Protocol

Cell Culture:
Plate cells in a 96-well plate and incubate for required time period depending upon the cell type. Treat cells with desired test compound(s) for 1-72 hrs.

BrdU Incorporation:
Add 10X BrdU solution into desired wells to a final concentration of 1X. Incubate plate at 37°C for 1-4 hrs.

Δ Note: *Seed cells at a density of 2500-10,000 cells/well depending on the cell growth rate. Incubation time needs to be optimized for each cell line.*

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BrdU Detection:

1. Remove medium from cells & add 100 µl of Fixing/Denaturing Solution into each well. Incubate at room temperature for 30 min.
2. Remove solution carefully & add 100 µl of 1X BrdU Detection Antibody solution into each well. Incubate at room temperature for 1 hr with gentle shaking.
3. Remove solution & wash wells with 300 µl 1X Wash Buffer (2 times).
4. After washing, add 100 µl of 1X Anti-mouse HRP-linked Antibody Solution into each well and incubate the plate at room temperature for 1 hr. Remove solution and wash wells with 300 µl of 1X Wash Buffer (3 times).

Measurement

Add 100 µl TMB Substrate into each well & measure the absorbance at 650 nm for 5-30 min. at room temperature to monitor the color development. To stop the color development, add 100 µl Stop Solution into each well & measure absorbance at 450 nm.

Δ Notes:

a) For suspension cells, centrifuge plate at 300 x g for 10 min. and remove medium carefully before adding Fixing/Denaturing Solution.

b) Incubation time after addition of TMB substrate must be optimized to avoid over development of colour. Recommended absorbance is ~0.8-1. After addition of stop solution, read plate immediately.

c) The value from OD 450 nm will be roughly double of that from OD 650 nm.