BrdU Cell Proliferation Assay Kit #6813

Protocol

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A. Reagent Preparation

1. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each BrdU ELISA Kit) in purified water.
2. Prepare 1X detection antibody solution by diluting BrdU Detection Antibody 1:100 with Detection Antibody Diluent (green).
3. Prepare 1X HRP-conjugated secondary antibody solution by diluting Anti-mouse IgG, HRP-linked Antibody 1:100 with HRP-linked Antibody Diluent (red).
4. Prepare 10X BrdU solution by diluting BrdU 1:100 with cell culture medium.

B. BrdU Incorporation

1. Plate cells in 96-well plate and incubate with respective test substance. Typical seed cell number is 2500–100000 cells/well depending on cell growth rate. Typical incubation time is 1–72 hr.
2. Add prepared 10X BrdU solution to plate wells, for a final 1X concentration. (Example: For 100 µl medium in the plate, add 10 µl of 10X BrdU solution per well.)
3. Place cells in incubator. Typical incubation time is 1–24 hr.
4. Remove medium. For suspension cells, centrifuge the plate at 300 g for 10 min, then remove medium.

C. BrdU Assay

1. Add 100 µl/well of the Fixing/Denaturing Solution, keep the plate at room temperature for 30 min. Remove solution.
2. Add 100 µl/well prepared 1X detection antibody solution, keep plate at room temperature for 1 hr. Remove solution and wash plate 3 times with 1X Wash Buffer.
3. Add 100 µl/well prepared 1X HRP-conjugated secondary antibody solution, keep plate at room temperature for 30 min. Remove the solution and wash plate 3 times with 1X Wash Buffer.
4. Add 100 µl TMB Substrate (#7004 (/product/productDetail.jsp?productId=7004)).
5. Incubate for 30 min at room temperature.

   NOTE: Watch the color change as it may be necessary to stop the reaction prior to the standard development time of 30 min.

6. Add 100 µl STOP Solution (#7002 (/product/productDetail.jsp?productId=7002)).
7. Read absorbance at 450 nm (For optimal readings, read the plate within 30 min of adding STOP Solution).

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