Product Manual

# CytoSelect™ EdU Competitive ELISA Kit

**Catalog Number**

**CBA-5101** **96 assays**

**FOR RESEARCH USE ONLY**
**Not for use in diagnostic procedures**

CELL BIOLABS, INC.
*Creating Solutions for Life Science Research*

## Introduction

Ethynyldeoxyuridine (EdU), like bromodeoxyuridine (BrdU), is a synthetic analog of the natural nucleoside thymidine. EdU is commonly used in the detection of proliferating cells in living tissues. Introduction of EdU to cells results in uptake and incorporation into the newly synthesized DNA of replicating cells in place of thymidine. Modified fluorescent conjugates specific for EdU are then typically used to detect the incorporated EdU.

The CytoSelect™ EdU Competitive ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of EdU in DNA samples. The quantity of EdU in unknown samples is determined by comparing its absorbance with that of a known EdU standard curve. The kit has detection sensitivity limit of ~ 800 ng/mL EdU. Each CytoSelect™ EdU Competitive ELISA Kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

## Assay Principle

As opposed to most cell-based thymidine analog assay kits that can only measure qualitative differences in incorporation, the CytoSelect™ EdU Competitive ELISA kit is a competitive ELISA for the quantitative measurement of EdU. The unknown EdU-containing samples or free EdU standards are first added to an EdU conjugate preabsorbed microplate. After a brief incubation, an anti-EdU monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The EdU content in unknown samples is determined by comparison with a predetermined EdU standard curve.

## Related Products

1. CBA-081: CytoSelect™ 96-Well Anoikis Assay
2. CBA-240: CytoSelect™ Cell Viability and Cytotoxicity Assay
3. CBA-250: CytoSelect™ Cell Proliferation Assay Reagent (Fluorometric)
4. CBA-251: CytoSelect™ BrdU Cell Proliferation ELISA Kit
5. CBA-252: CytoSelect™ MTT Cell Proliferation Assay Reagent
6. CBA-253: CytoSelect™ WST-1 Cell Proliferation Assay Reagent
7. CBA-254: CytoSelect™ Proliferating Cell Nuclear Antigen (PCNA) ELISA Kit
8. CBA-5098: CytoSelect™ BrdU Competitive ELISA Kit
9. CBA-5100: CytoSelect™ IdU Competitive ELISA Kit

## Kit Components

### Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-EdU Antibody (Part No. 51011C): One 10 µL vial.
3. Secondary Antibody, HRP Conjugate (Part No. 230003): One 20 µL vial.

4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.
8. EdU Standard (Part No. 51012C): One 125 µL vial of 1 mg/mL Ethynyldeoxyuridine.

**Box 2 (shipped on blue ice packs)**

1. EdU Conjugate (100X) (Part No. 51013D): One 100 µL vial.
2. 100X Conjugate Diluent (Part No. 281603): One 300 µL vial.

## Materials Not Supplied

1. EdU containing samples such as digested DNA extracted from cells or tissues
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
6. DNA extraction kit
7. Nuclease free water
8. Nuclease P1
9. Alkaline Phosphatase

## Storage

Upon receipt, aliquot and store EdU standard at -20°C and the EdU Conjugate (100X) at -80ºC avoiding multiple freeze/thaw cycles. Store all other components at 4ºC.

## Preparation of Reagents

- EdU Conjugate Coated Plate:

  *Note: The EdU Conjugate coated wells are not stable and should be used within 24 hrs after coating. Only coat the number of wells to be used immediately.*

  1. Immediately before use, prepare 1X Conjugate Diluent by diluting the 100X Conjugate Diluent in 1X PBS. Example: Add 50 µL to 4.95 mL of 1X PBS.
  2. Immediately before use, prepare 1X EdU Conjugate by diluting the 100X EdU Conjugate in 1X Conjugate Diluent. Example: Add 50 µL of 100X EdU Conjugate to 4.950 mL of 1X Conjugate Diluent.
  3. Add 100 µL of the 1X EdU Conjugate to each well to be tested and incubate 37ºC for two hours or overnight at 4ºC. Remove the EdU Conjugate coating solution and wash twice with 1X PBS. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to

each well and block for 1 hr at room temperature on an orbital shaker. Transfer the plate to 4ºC and remove the Assay Diluent **immediately before use.**

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-EdU Antibody and Secondary Antibody: Immediately before use dilute the Anti-EdU Antibody 1:500 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

## Preparation of Standard Curve

1. Use the provided stock EdU Standard 1 mg/mL solution to prepare a series of the remaining EdU standards according to Table 1 below.

| Standard Tubes | 1 mg/mL EdU Standard (µL) | Assay Diluent (µL) | EdU (µg/mL) | EdU (µM) |
|---|---|---|---|---|
| 1 | 25 | 475 | 50 | 198 |
| 2 | 250 of Tube #1 | 250 | 25 | 99 |
| 3 | 250 of Tube #2 | 250 | 12.5 | 49.5 |
| 4 | 250 of Tube #3 | 250 | 6.25 | 24.8 |
| 5 | 250 of Tube #4 | 250 | 3.13 | 12.4 |
| 6 | 250 of Tube #5 | 250 | 1.56 | 6.2 |
| 7 | 250 of Tube #6 | 250 | 0.78 | 3.1 |
| 8 | 0 | 250 | 0 | 0 |

**Table 1. Preparation of EdU Standards.**

## Preparation of DNA Samples

1. Extract DNA from cell or tissue samples that have incorporated exogenous EdU by a desired method or commercial DNA Extraction kit.
2. Dissolve extracted DNA in water at 1-5 mg/mL.
3. Convert DNA sample to single-stranded DNA by incubating the sample at 95ºC for 5 minutes and rapidly chilling on ice.
4. Digest DNA sample to nucleosides by incubating the denatured DNA with 5-20 units of nuclease P1 (previously reconstituted in the manufacturer's recommended buffer) for 2 hrs at 37ºC in a final concentration of 20 mM Sodium Acetate, pH 5.2.
5. Add 5-10 units of alkaline phosphatase (previously reconstituted in the manufacturer's recommended buffer) plus sufficient Tris buffer to a final concentration of 100 mM Tris, pH 7.5, and incubate for 1 hr at 37ºC.
6. Centrifuge the reaction mixture for 5 minutes at 6000 x g and collect the supernatant for use in the ELISA.

## Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each EdU sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or EdU standards to the wells of the EdU Conjugate coated plate. Incubate at room temperature for 5 minutes on an orbital shaker.
3. Add 50 µL of the diluted anti-EdU antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Secondary Antibody-HRP Enzyme Conjugate to all wells.
6. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash microwell strips 3 times according to step 4 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   *Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.*
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

## Example of Results

The following figures demonstrate typical EdU Competitive ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

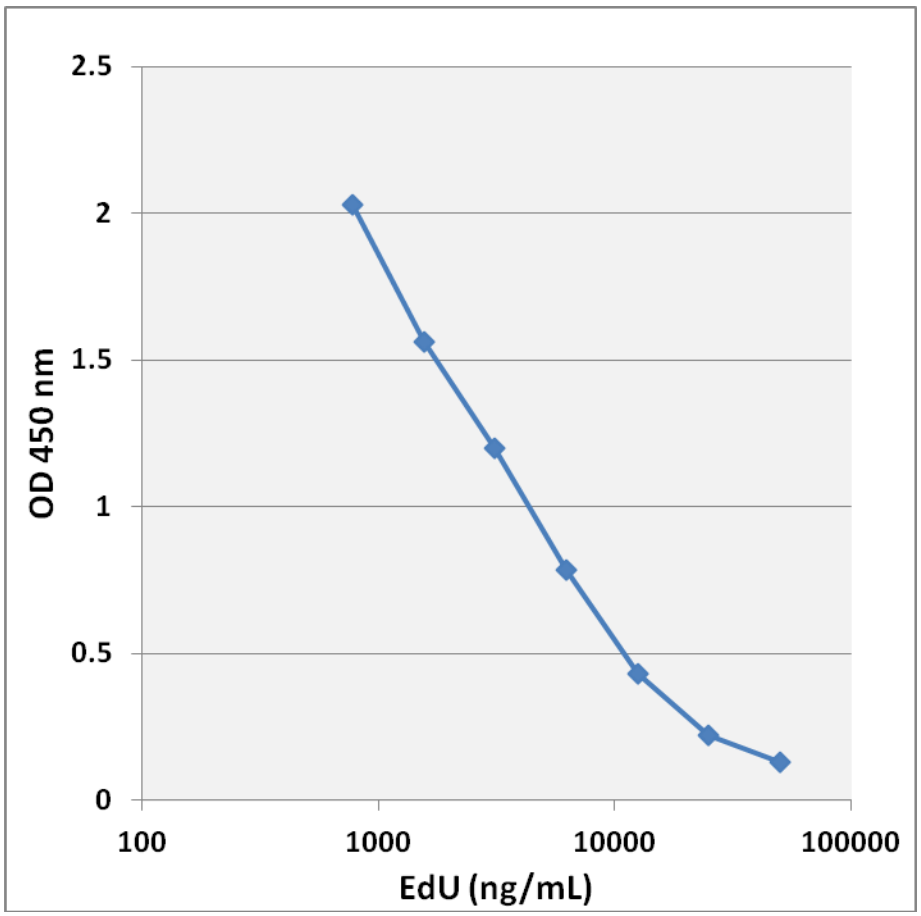


**Figure 1: EdU ELISA Standard Curve.**

## References

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## Warranty

In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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