Product Manual

CytoSelect™ IdU Competitive ELISA Kit

Catalog Number

CBA-5100 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Iododeoxyuridine (IdU), like bromodeoxyuridine (BrdU), is a synthetic analog of the natural nucleoside thymidine. IdU is commonly used in the detection of proliferating cells in living tissues. Introduction of IdU to cells results in uptake and incorporation into the newly synthesized DNA of replicating cells in place of thymidine. Antibodies specific for IdU can then be used to detect the incorporated IdU.

The CytoSelect™ IdU Competitive ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of IdU in DNA samples. The quantity of IdU in unknown samples is determined by comparing its absorbance with that of a known IdU standard curve. The kit has detection sensitivity limit of 80 ng/mL IdU. Each CytoSelect™ IdU 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™ IdU Competitive ELISA kit is a competitive ELISA for the quantitative measurement of IdU. The unknown IdU-containing samples or free IdU standards are first added to an IdU conjugate preadsorbed microplate. After a brief incubation, an anti-IdU monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The IdU content in unknown samples is determined by comparison with a predetermined IdU 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-5101: CytoSelect™ EdU 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-IdU Antibody (500X) (Part No. 51001C): 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. IdU Standard (Part No. 51002C): One 25 µL vial of 0.5 mg/mL Iododeoxyuridine.

Box 2 (shipped on blue ice packs)

1. IdU Conjugate (100X) (Part No. 51003D): One 100 µL vial.
2. 100X Conjugate Diluent (Part No. 281603): One 300 µL vial.

Materials Not Supplied

1. IdU 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 IdU standard at -20°C and the IdU Conjugate (100X) at -80ºC avoiding multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- IdU Conjugate Coated Plate:

  Note: The IdU 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 IdU Conjugate by diluting the 100X IdU Conjugate in 1X Conjugate Diluent. Example: Add 50 µL of 100X IdU Conjugate to 4.950 mL of 1X Conjugate Diluent.
  3. Add 100 µL of the 1X IdU Conjugate to each well to be tested and incubate at 37ºC for two hours or overnight at 4ºC. Remove the IdU 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-IdU Antibody and Secondary Antibody: Immediately before use dilute the Anti-IdU 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 IdU Standard 0.5 mg/mL solution to prepare a series of the remaining IdU standards according to Table 1 below.

Standard Tubes	0.5 mg/mL IdU Standard (µL)	Assay Diluent (µL)	IdU (ng/mL)	IdU (µM)
1	5	495	5000	14.1
2	250 of Tube #1	250	2500	7.06
3	250 of Tube #2	250	1250	3.53
4	250 of Tube #3	250	625	1.77
5	250 of Tube #4	250	313	0.88
6	250 of Tube #5	250	156	0.44
7	250 of Tube #6	250	78	0.22
8	0	250	0	0

Table 1. Preparation of IdU Standards.

Preparation of DNA Samples

1. Extract DNA from cell or tissue samples that have incorporated exogenous IdU 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 IdU sample including unknown and standard should be assayed in duplicate.
2. Add 50 µL of unknown sample or IdU standards to the wells of the IdU Conjugate coated plate. Incubate at room temperature for 5 minutes on an orbital shaker.
3. Add 50 µL of the diluted anti-IdU 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 IdU ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

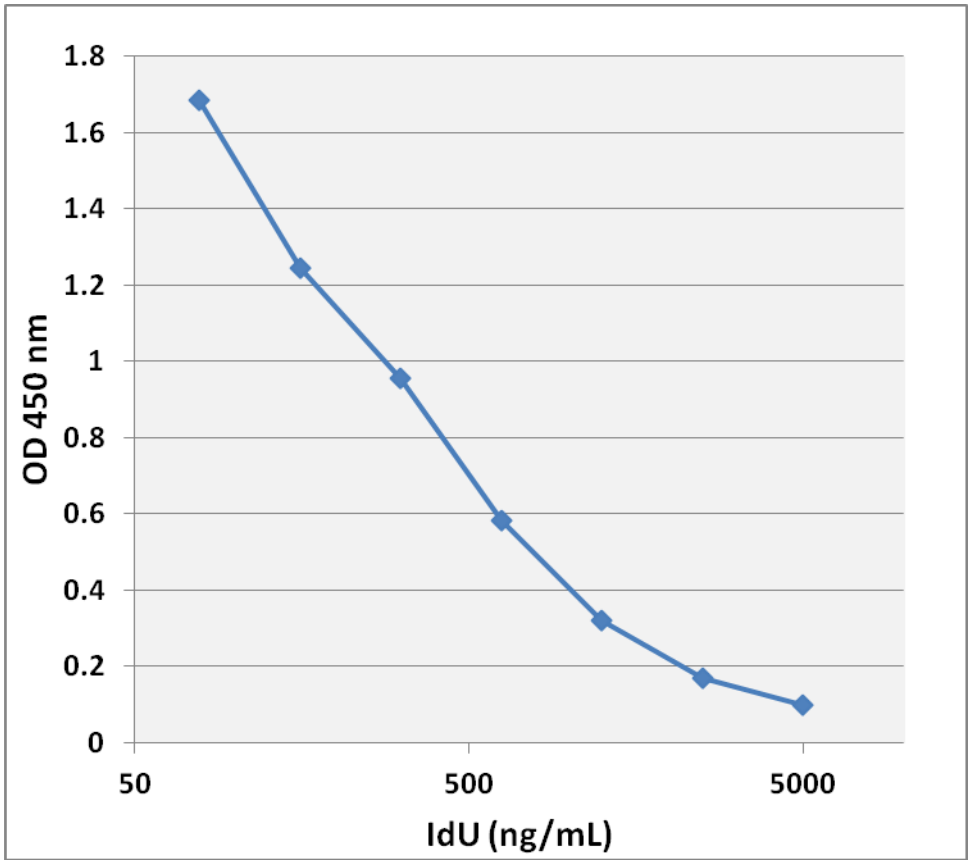

Figure 1: IdU ELISA Standard Curve.

References

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3. Gambetta Hampon E and Eidinoff ML. (1961) *Canc. Res*. **21**:345-352.
4. Tuttle AH, Rankin MM, Teta M, Sartori DJ, Stein GM, Kim GJ, Virgilio C, Granger A, Zhou D, Long SH, Schiffman AB, Kushner JA. (2010) *J. Vis. Exp*. **46**:1-5.

Warranty

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