Product Manual

CytoSelect™ Proliferating Cell Nuclear Antigen (PCNA) ELISA Kit

Catalog Number

CBA-254 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Proliferating Cell Nuclear Antigen (PCNA) was originally identified as an auxiliary factor for DNA polymerase δ, and has been described as a DNA sliding clamp that acts as a polymerase processivity factor. PCNA aids in processivity through association with various DNA replication associated proteins. In addition to DNA replication, PCNA function is associated with chromatin remodeling, sister-chromatid cohesion and cell cycle control. PCNA is often used as a marker for cell proliferation.

In addition to its role in DNA replication and proliferation, PCNA plays a role in DNA repair. PCNA activates the DNA damage tolerance pathway in two ways. First, PCNA promotes translesion synthesis through DNA polymerase ζ (zeta), whereby DNA replication stalled at a damaged site can be essentially bypassed, ultimately leaving a mutation behind for the sake of cell survival. Secondly, the PCNA promotes the "template switch" pathway where the homologous recombination machinery is recruited to bypass DNA damage. PCNA itself is post-translationally modified with ubiquitin which activates one or the other DNA damage tolerance pathway: mono-ubiqutination of lysine 164 activates translesion synthesis, while further polyubiquitination at lysine 63 favors the "template switch" pathway.

Cell Biolabs' CytoSelect™ Proliferating Cell Nuclear Antigen (PCNA) ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of PCNA from nuclear and whole cell extracts. The kit detects PCNA from mouse, rat and human, and has a detection sensitivity limit of 12.5 ng/mL PCNA. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

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

Kit Components

Box 1 (shipped at room temperature)

1. Anti-PCNA Antibody Coated Plate (Part No. 125401): One 96-well strip plate (8 x 12).
2. Anti-PCNA Antibody (1000X) (Part No. 125402): One 12 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.

Box 2 (shipped on blue ice packs)

1. PCNA Standard (Part No. 125403): One 80 µL vial of 20 µg/mL recombinant human PCNA Protein

Materials Not Supplied

1. Mammalian cells, cell extraction kits or reagents
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store recombinant PCNA Standard at -80ºC and avoid freeze/thaw. Store all other components at 4ºC.

Preparation of Reagents

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

Preparation of human PCNA Standards

Prepare a dilution series of PCNA standards in the concentration range of 0 to 100 ng/mL in Assay Diluent (Table 1).

Standard Tubes	20 µg/mL PCNA Standard (µL)	Assay Diluent (µL)	PCNA (ng/mL)
1	16	784	400
2	400 of Tube #1	400	200
3	400 of Tube #2	400	100
4	400 of Tube #3	400	50
5	400 of Tube #4	400	25
6	400 of Tube #5	400	12.5
7	400 of Tube #6	400	6.25
8	0	400	0

Table 1. Preparation of PCNA Standards.

Assay Protocol

1. Prepare cell or tissue whole extracts in RIPA Lysis Buffer (25 mM Tris pH 7.6, 150 mM NaCl, 1% Triton X-100 or NP-40, 1% Sodium Deoxycholate, 0.1% SDS, Proteinase Inhibitors (please refer to Cell Biolabs Cat. No. AKR-190 for sample preparation) or nuclear extracts in desired methods.
2. Add 100 µL of PCNA unknown sample or standard to the Anti-PCNA Antibody Coated Plate. Each PCNA unknown sample, standard and blank should be assayed in duplicate.
3. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
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 Anti-PCNA antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
6. Wash the strip wells 3 times according to step 4 above.
7. Add 100 µL of the diluted Secondary Antibody HRP Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
8. Wash the strip wells 3 times according to step 4 above. Proceed immediately to the next step.
9. 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.
10. 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).
11. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate CytoSelect™ Proliferating Cell Nuclear Antigen (PCNA) ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

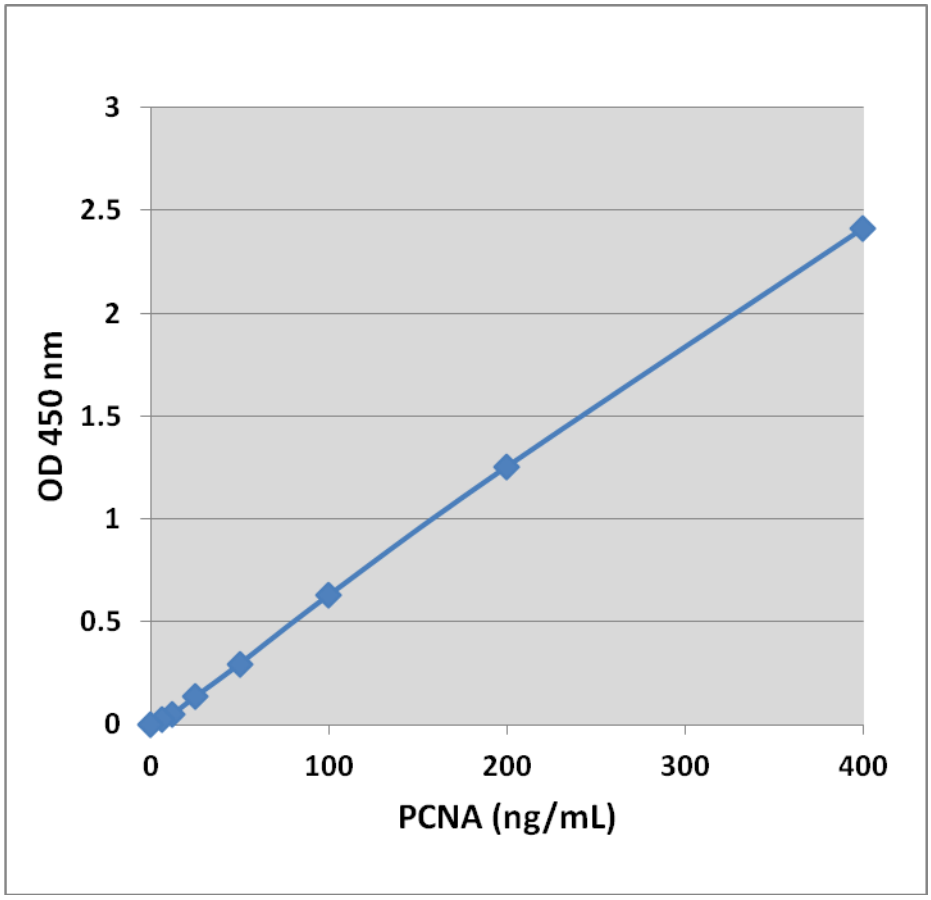

Figure 1: CytoSelect™ Proliferating Cell Nuclear Antigen (PCNA) Standard Curve.

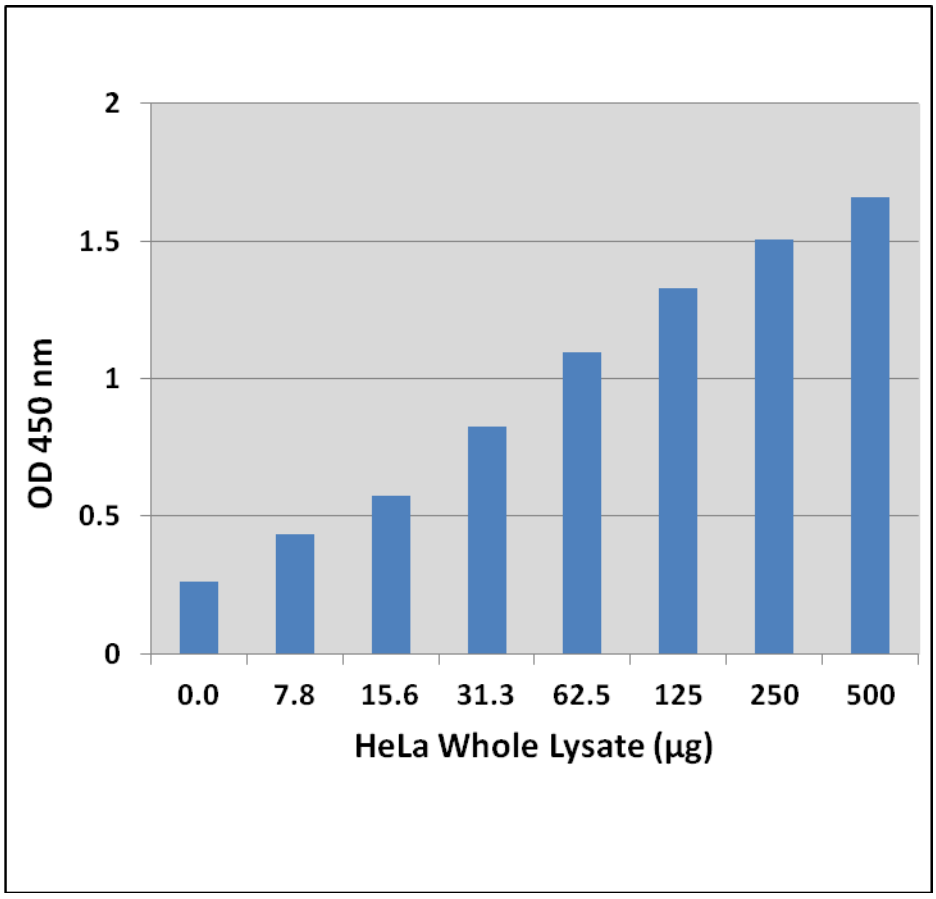

Figure 2: PCNA Detection in HeLa Whole Cell Lysate. Whole cell lysates from HeLa cells were prepared in RIPA Lysis Buffer and protein concentration was determined by BCA Protein assay.

References

1. Strzalka W., and Ziemienowicz A (2011) *Annals of Botany* **107**: 1127-1140
2. Zhang W, Qin Z, Zhang X, and Xiao W (2011) *Febs Lett.***585:** 2786-2794
3. Garg P, Stith CM, Majka J, and Burgers PMJ (2005) *J. Biol. Chem.* **280:** 23446-23450
4. Guzinska-Ustymowicz K, Pryczynicz A, Kemona A, and Czyzewska J (2009) *Anticancer Res.* **29:**3049-3052.

Recent Product Citations

1. Chow, A. et al. (2020). Dasatinib inhibits peripapillary scleral myofibroblast differentiation. *Exp Eye Res*. doi: 10.1016/j.exer.2020.107999.
2. Guo, J.W. et al. (2020). Salvianolic Acid B in Microemulsion Formulation Provided Sufficient Hydration for Dry Skin and Ameliorated the Severity of Imiquimod-Induced Psoriasis-Like Dermatitis in Mice. *Pharmaceutics*. **12**(5):E457. doi: 10.3390/pharmaceutics12050457.
3. Pitha, I. et al. (2018). Rho-Kinase Inhibition Reduces Myofibroblast Differentiation and Proliferation of Scleral Fibroblasts Induced by Transforming Growth Factor β and Experimental Glaucoma. *Transl Vis Sci Technol*. **7**(6):6. doi: 10.1167/tvst.7.6.6.

4. Han, Z., et al. (2017). Therapeutic value of nerve growth factor in promoting neural stem cell survival and differentiation and protecting against neuronal hearing loss. *Mol Cell Biochem*. **428**(1-2):149-159doi:10.1007/s11010-016-2925-5.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com