# abcam

## Product datasheet

## Human p21 ELISA Kit ab214658

Recombinant SimpleStep ELISA

2 References 6 Images

Overview

Recovery

**Product name** Human p21 ELISA Kit

**Detection method** Colorimetric

**Precision** 

Sample	n	Mean	SD	CV%
MCF7 lysates	5			4.2%

Inter-assay

Sample specific recovery

Intra-assav

Sample	n	Mean	SD	CV%
MCF7 lysates	3			7.3%

Sample type Cell culture extracts, Tissue Lysate

Assay type Sandwich (quantitative)

Sensitivity 11 pg/ml

62.5 pg/ml - 4000 pg/ml Range

Sample type	Average %	Range
Cell culture media	120	115% - 129%

Assay time 1h 30m

**Assay duration** One step assay

Species reactivity Reacts with: Human

**Product overview** Human p21 ELISA Kit (ab214658) is a single-wash 90 min sandwich ELISA designed for the

quantitative measurement of p21 protein in tissue lysate and cell culture extracts. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human p21 with 11 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This

approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (<u>ab203359</u>) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

p21 plays a critical role in the cellular response to DNA damage, and its overexpression results in cell cycle arrest. Upregulation of p21 mRNA and protein following ionizing radiation is dependent on p53 (TP53), and p21 mediates cell cycle arrest in response to the p53 checkpoint pathway. p21 binds to and inhibits cyclin-dependent kinase activity, preventing phosphorylation of critical cyclin-dependent kinase substrates and blocking cell cycle progression. p21 functions in the nuclear localization and assembly of cyclin D-CDK4 complex and promotes its kinase activity towards RB1. At higher stoichiometric ratios, p21 inhibits the kinase activity of the cyclin D-CDK4 complex.

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

**Platform** 

**Notes** 

Microplate

#### **Properties**

## Storage instructions

Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
10X Human p21 Capture Antibody	1 x 600µl
10X Human p21 Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent 5BI	1 x 6ml
Human p21 Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 12ml

Components	1 x 96 tests
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

#### **Function**

May be the important intermediate by which p53/TP53 mediates its role as an inhibitor of cellular proliferation in response to DNA damage. Binds to and inhibits cyclin-dependent kinase activity, preventing phosphorylation of critical cyclin-dependent kinase substrates and blocking cell cycle progression. Functions in the nuclear localization and assembly of cyclin D-CDK4 complex and promotes its kinase activity towards RB1. At higher stoichiometric ratios, inhibits the kinase activity of the cyclin D-CDK4 complex.

#### Tissue specificity

Expressed in all adult human tissues, with 5-fold lower levels observed in the brain.

## Sequence similarities

Belongs to the CDI family.

**Domain** 

The PIP-box K+4 motif mediates both the interaction with PCNA and the recuitment of the DCX(DTL) complex: while the PIP-box interacts with PCNA, the presence of the K+4 submotif, recruits the DCX(DTL) complex, leading to its ubiquitination.

The C-terminal is required for nuclear localization of the cyclin D-CDK4 complex.

recruit the DCX(DTL) complex, leading to its degradation.

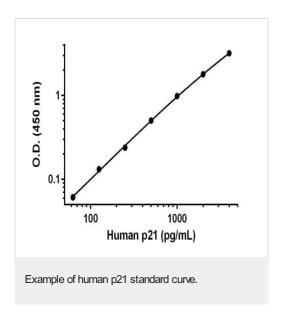
Post-translational modifications

Phosphorylation of Thr-145 by Akt or of Ser-146 by PKC impairs binding to PCNA. Phosphorylation at Ser-114 by GSK3-beta enhances ubiquitination by the DCX(DTL) complex. Ubiquitinated by MKRN1; leading to polyubiquitination and 26S proteasome-dependent degradation. Ubiquitinated by the DCX(DTL) complex, also named CRL4(CDT2) complex, leading to its degradation during S phase or following UV irradiation. Ubiquitination by the DCX(DTL) complex is essential to control replication licensing and is PCNA-dependent: interacts with PCNA via its PIP-box, while the presence of the containing the 'K+4' motif in the PIP box,

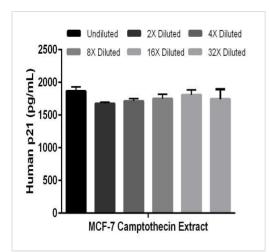
#### Cellular localization

Cytoplasm. Nucleus.

## **Images**

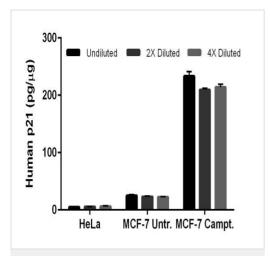


Background-subtracted data values (mean +/- SD) are graphed.



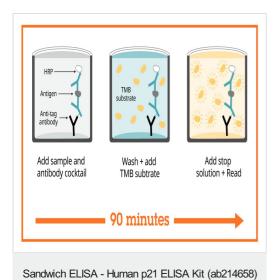
Interpolated concentration of native p21 in human extract samples of MCF-7 cells treated for 18 hours with 1 ?M camptothecin based on 8  $\mu$ g/mL extract load

The concentrations of p21 were measured in duplicates in a two-fold dilution series and interpolated from the p21 standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean p21 concentration was determined to be 1,811 pg/mL in extract sample of MCF-7 cells treated for 18 hours with 1 mM camptothecin.



. Interpolated concentrations of native p21 in human extract samples.

Interpolated concentrations of native p21 in human extract samples of HeLa cells, vehicle (DMSO)-treated MCF-7 cells and 18 hours 1  $\mu$ M camptothecin-treated MCF-7 cells samples. The concentrations of p21 were measured in duplicates at 3 different dilutions, interpolated from the p21 standard curve. Note that Undiluted HeLa cell extract and Undiluted vehicle (DMSO)-treated MCF-7cell extract samples were at 32  $\Box$ g/mL. Note that Undiluted 18 hours 1  $\Box$ M camptothecin-treated MCF-7 cells samples were at 8  $\mu$ g/mL. The interpolated, dilution factor-corrected values are graphed in pg of p21 per  $\mu$ g of total protein (mean +/- SD, n=2).



SimpleStep ELISA technology allows the formation of the antibodyantigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



To learn more about the advantages of recombinant antibodies see **here**.



To learn more about the advantages of SimpleStep  $\mathsf{ELISA}^{@}$  kits see  $\underline{\mathsf{here}}$ .

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