abcam

Product datasheet

Mouse p21 ELISA Kit (CDKN1A) ab205576

Recombinant SimpleStep ELISA

1 References 5 Images

Overview

Product name Mouse p21 ELISA Kit (CDKN1A)

Detection method Colorimetric

Precision Intra-assav

Sample	n	Mean	SD	CV%
Cell extract	5			3.4%

Inter-assay

Sample	n	Mean	SD	CV%
Cell extract	3			7.8%

Sample type Cell culture extracts, Tissue Extracts

Assay type Sandwich (quantitative)

Sensitivity 2.7 pg/ml

Range 62.5 pg/ml - 4000 pg/ml

Assay time 1h 30m

Assay duration One step assay

Species reactivity Reacts with: Mouse

Product overview Mouse p21 ELISA Kit (CDKN1A) (ab205576) is a single-wash 90 min sandwich ELISA designed

> for the quantitative measurement of p21 (CDKN1A) protein in cell culture extracts and tissue extracts. It uses our proprietary SimpleStep ELISA® technology. Quantitate Mouse p21

(CDKN1A) with 2.7 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 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. p21 may be the important intermediate by which p53/TP53 mediates its role as an inhibitor of cellular proliferation in response to DNA damage.

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

Pre-coated microplate (12 x 8 well strips)

Properties

Storage instructions

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

Components	1 x 96 tests
10X Mouse p21 Capture Antibody	1 x 600µl
10X Mouse 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 5BR	1 x 6ml
Mouse p21 Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 12ml
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.

Post-translational modifications

Phosphorylation of Thr-145 by Akt or of Ser-146 by PKC impairs binding to PCNA.

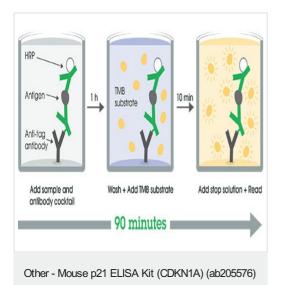
Phosphorylation at Ser-114 by GSK3-beta enhances ubiquitination by the DCX(DTL) complex. Ubiquitinated by MKRN1; leading to polyubiquitination and 26S proteasome-dependent degradation. Ubiquitinated by the DCX(DTL) complex, also named CRL4(CDT2) complex, leading to its degradation during S phase or following 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, recruit the DCX(DTL) complex, leading to its degradation.

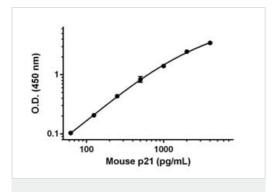
Cellular localization

Cytoplasm. Nucleus.

Images

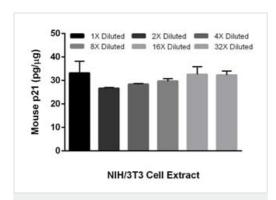


SimpleStep ELISA technology allows the formation of the antibodyantigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



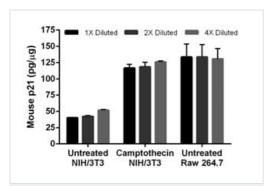
Example of p21 standard curve.

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



Interpolated concentrations of p21 in mouse NIH/3T3 cell extract samples.

The concentrations of p21 were measured in duplicates, interpolated from the p21 standard curves and corrected for sample dilution. Note that 1X Diluted NIH/3T3 cell extract samples were at 80 μ g/mL. The interpolated, dilution factor-corrected values are plotted in pg of p21 per μ g of total protein (mean +/- SD, n=2).



Camptothecin treatment of NIH/3T3 cells induces expression of p21.

NIH/3T3 cells were cultured in the absence or presence of 1 μ M camptothecin for 18 hours. Raw 264.7 cells were cultured in the absence of camptothecin. The cell extracts were prepared. The concentrations of p21 were measured in the diluted cell extracts in duplicates, interpolated from the p21 standard curves and corrected for sample dilution. Note that 1X Diluted untreated NIH/3T3 cell extract samples were at 44 μ g/mL. Note that 1X Diluted camptothecin treated NIH/3T3 cell extract samples were at 11 μ g/mL. Note that 1X Diluted untreated Raw 264.7 cell extract samples were at 6 μ g/mL. The interpolated, dilution factor-corrected values are plotted in pg of p21 per μ g of total protein (mean +/- SD, n=2).







Long-term and scalable supply Recombinant



specificity



Sandwich ELISA - Mouse p21 ELISA Kit (CDKN1A) (ab205576)

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

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