abcam

Product datasheet

Anti-p21 (phospho T145) antibody ab47300

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Overview

Product name	Anti-p21 (phospho T145) antibody
Description	Rabbit polyclonal to p21 (phospho T145)
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, WB, IHC-P, ELISA
Species reactivity	**Reacts with:** Human
Immunogen	synthesized phosphopeptide derived from human p21Cip1 around the phosphorylation site of threonine 145 (R-Q-TP-S-M)
Positive control	Human breast carcinoma tissue and EGF treated HeLa cell extracts
General notes	p21Cip1 (phospho-Thr145) antibody detects endogenous levels of p21Cip1 only when phosphorylated at threonine 145.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 50% Glycerol, 0.87% Sodium chloride
Purity	Immunogen affinity purified
Purification notes	The antibody was purified using epitope-specific phosphopeptide. The antibody against non-phosphopeptide was removed by chromatography using non-phosphopeptide corresponding to the phosphorylation site.
Primary antibody notes	p21Cip1 (phospho-Thr145) antibody detects endogenous levels of p21Cip1 only when phosphorylated at threonine 145.
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab47300** in the following tested applications.

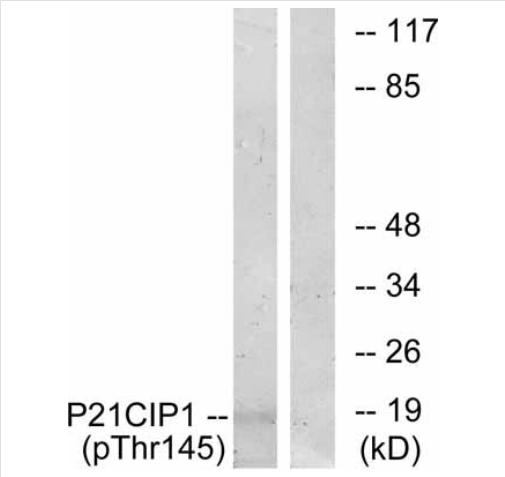
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		Use a concentration of 1 µg/ml.
WB		1/500 - 1/1000. Detects a band of approximately 32 kDa (predicted molecular weight: 18 kDa).
IHC-P	★★★★☆	Use at an assay dependent concentration.
ELISA		1/10000.

Target

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

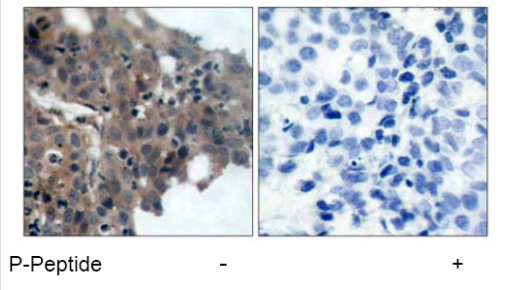


Western blot - Anti-p21 (phospho T145) antibody (ab47300)

All lanes : Anti-p21 (phospho T145) antibody (ab47300)

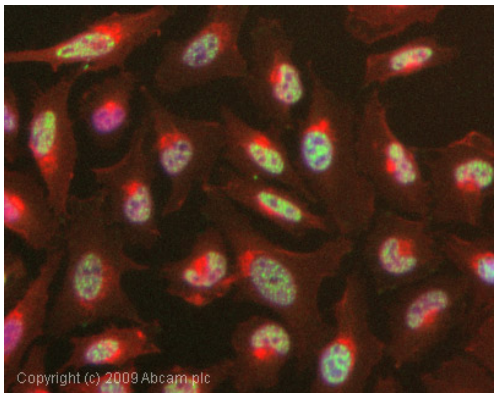
Lane 1 : EGF treated HeLa cells
Lane 2 : HeLa cells

Predicted band size: 18 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p21 (phospho T145) antibody (ab47300)

ab47300 staining human breast carcinoma tissue by IHC-P (left hand panel). The right hand panel shows staining in the presence of phospho-peptide.



Immunocytochemistry/ Immunofluorescence - Anti-p21 (phospho T145) antibody (ab47300)

ICC/IF image of ab47300 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab47300, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

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