Datasheet for ABIN4902067

anti-p53 antibody (meLys370)

1 Validation

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Overview

Quantity:	100 µL
Target:	p53 (TP53)
Binding Specificity:	meLys370
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This p53 antibody is un-conjugated
Application:	Western Blotting (WB), ELISA

Product Details

Purpose:	Rabbit polyclonal to p53 (Mono Methyl Lys370).
Immunogen:	Synthesized peptide derived from human p53 around the methylation site of K370.
Isotype:	IgG
Specificity:	Mono-Methyl-p53 (K370) Polyclonal Antibody detects endogenous levels of p53 around the methylation site of K370 protein.
Characteristics:	Rabbit Polyclonal to p53 (Mono Methyl Lys370).
Purification:	The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific immunogen.

Target Details

Target:	p53 (TP53)
Alternative Name:	p53 (TP53 Products)
Gene ID:	7157
UniProt:	P04637
Pathways:	p53 Signaling, MAPK Signaling, PI3K-Akt Signaling, Apoptosis, AMPK Signaling, Chromatin Binding, ER-Nucleus Signaling, Positive Regulation of Endopeptidase Activity, Hepatitis C, Protein targeting to Nucleus, Autophagy, Warburg Effect

Application Details

Application Notes:	WB 1:500-1:2000 ELISA 1:10000
Comment:	Ubiquitous. Isoforms are expressed in a wide range of normal tissues but in a tissue-dependent manner. Isoform 2 is expressed in most normal tissues but is not detected in brain, lung, prostate, muscle, fetal brain, spinal cord and fetal liver. Isoform 3 is expressed in most normal tissues but is not detected in lung, spleen, testis, fetal brain, spinal cord and fetal liver. Isoform 7 is expressed in most normal tissues but is not detected in prostate, uterus, skeletal muscle and breast. Isoform 8 is detected only in colon, bone marrow, testis, fetal brain and intestine. Isoform 9 is expressed in most normal tissues but is not detected in brain, heart, lung, fetal liver, salivary gland, breast or intestine.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	1 mg/mL
Buffer:	Liquid in PBS containing 50 % glycerol, 0.5 % BSA and 0.02 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Handling Advice:	Avoid repeated freeze/thaw cycles.
Storage:	-20 °C

Handling

Storage Comment:	Store at -20°C, and avoid repeat freeze-thaw cycles.

Successfully validated (Western Blotting (WB))

by Department of Prevention and Therapy of Chronic Diseases, Institute for Advanced Biosciences, CNRS UMR5309

Report Number: 101268

Date: Aug 03 2017

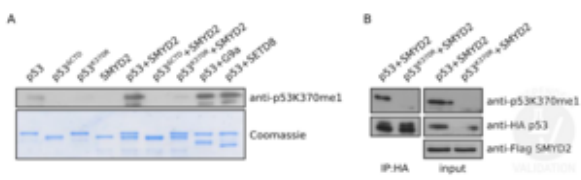
Target:	p53K370me
Lot Number:	B1001
Method validated:	Western Blotting (WB)
Positive Control:	p53, in vitro specifically p53K370me1 methylated using methyltransferase SMYD2 ectopic SMYD-methylated wildtype p53 from 293T cell extracts
Negative Control:	p53, in vitro methylated using methyltransferases G9a and SETD8 $p53^{\Delta CTD}$, lacking C-terminal domain containing known methylable lysines $p53^{K370R}$, lacking the lysine 370 methylation site, mutated into arginine
Notes:	Passed. ABIN4902067 specifically recognizes monomethylated p53K370me1 and to a lesser extent p53Kme1.
Primary Antibody:	ABIN4902067
Secondary Antibody:	HRP-conjugated goat anti-rabbit or anti-mouse antibody (Biorad)
Protocol:	• In vitro p53 methylation assay: ◦ Clone SMYD2 and p53 from human cDNA library into pGex6p1 vector. ◦ Perform p53 mutagenesis using QuickChange II site-directed mutagenesis kit (Agilent, 200523). ◦ Transform vectors into BL21 pLys bacteria (Agilent) and induced protein expression with 0.1mM IPTG in 250mL culture ON at 19°C. ◦ Purify proteins by GST pulldown and quantify using Bradford assay and Coomassie gel staining. ◦ For the in vitro methylation assays incubate 1 to 2µg recombinant protein with 1µg of recombinant methyltransferases and 2µCi 3H-AdoMet (American Radiolabelled Chemicals) in buffer containing 50mM Tris-HCl pH8.0, 10% glycerol, 20mM KCl, 5mM MgCl2, and 1mMPMSF at ON at 30°C. ◦ Resolve the reaction mixture by SDS–PAGE, followed by autoradiography and Coomassie stain (Pierce). • "In cellulo" p53 methylation assay: ◦ Subclone SMYD2 and p53 constructs from pGex6p1 vectors into pcagFLAG (SMYD2) or pCDNA3.1HA (p53).

- Transfect plasmids into 293T cells using Lipofectamine 2000 (ThermoFisher) according to supplier's protocol.
- Harvest cells 36h post-transfection to allow methylation of ectopic p53 by SMYD2 in cells.

- Western blot:
  - Separate 20µg of each sample on freshly cast SDS-PAGE gel in a gel electrophoresis chamber (BioRad) for 1h at 140V and RT.
  - Transfer proteins onto ethanol-activated 0.2µm Hybond PVDF membrane (Amersham) with a semi-dry Trans-Blot Turbo (BioRad) for 30min at 20V.
  - Block the membrane using 5% milk in TBS-Tween 0.1% for 1h at RT.
  - Incubate the membrane with primary rabbit anti-p53 meLys370 antibody (antibodies-online, ABIN4902067, LOT B1001), mouse anti-HA antibody (Sigma), or mouse anti-Flag tag antibody (Sigma) diluted 1:1000 in 1% BSA TBS-Tween 0.1% ON at 4°C.
  - Wash the membrane 3x for 10min in TBS-Tween 0.1%.
  - Incubate the membrane with secondary HRP-conjugated goat anti-rabbit or anti-mouse antibody (Biorad) diluted 1:3000 in 1% BSA TBS-Tween 0.1% for 1h at RT.
  - Wash the membrane 3x for 10min in TBS-Tween 0.1%.
  - Visualize protein bands using ECL RevelBlot intense (Ozyme) and signal detection on Hyperfilm ECL 18 x24cm (Amersham).

Experimental Notes:

- Antibody ABIN4902067 recognizes greatly its specific substrate in vitro: it shows a strong signal when SMYD2 is present, implying monomethylation of K370. The signal is lost when using $p53^{K370R}$ and $p53^{\Delta CTD}$ mutants. However, ABIN4902067 also recognizes to a lesser extent in vitro other p53 methylation such as p53 K373me1/2 and p53K382me2 caused by G9a and SETD8 respectively.
- The antibody does recognize unmethylated p53 when using a large quantity of the recombinant protein (not shown).
- ABIN4902067 gives a very clean and specific signal when using ectopic p53 from 293T cell extract: only p53 methylated by SMYD2 is detected, not the $p53^{K370R}$ mutant. The antibody can even be used without an initial immunoprecipitation of total p53 as observable in our figure.
- The binding affinity of ABIN4902067 can be ordered as follows: p53K370me1>p53Kme1>>>p53.



Validation image no. 1 for anti-Tumor Protein P53 (TP53) (meLys370) antibody (ABIN4902067)

A. In vitro methylation assay of wt p53 and the $p53^{K370R}$ and $p53^{\Delta CTD}$ mutants by methyltransferases SMYD2, G9a, and SETD8 (upper panel). See experimental notes for details. The lower panel shows a Commassie stain of the gel prior to the immunoblot. B. Western blot of 293T cellular extracts ectopically expressing HA-tag wt p53 or $p53^{K370R}$ in the presence of DYKDDDDK-tagged SMYD2, verified using an DYKDDDDK-tag antibody. In the panels on the left p53 in the lysates was enriched using an HA-tag antibody. WB with an HA-tag antibody reveals immunoprecipitated wt p53 and $p53^{K370R}$ whereas ABIN4902067 reveals specifically SMYD2-methylated wt p53.