

Anti-Mouse p53 Acetylated Lys 379 Polyclonal Antibody

Cat #2370-PC-050

Application guide

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

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Anti-acetylated-p53 Antibodies

Application Guide

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I. Introduction

The tumor suppressor p53 plays a key role in DNA damage and repair, apoptosis and cell growth. A variety of genotoxic stresses lead to stabilization and activation of p53 that are mediated by multiple post-translational modifications. The N-terminus of p53 may be heavily phosphorylated, whereas the C-terminus may be phosphorylated, acetylated or sumoylated. *In vivo* acetylation of the C-terminus of p53 by p300/CBP at Lysine (K) 372, K373, K381, and K382, and *in vitro* by pCAF at K320, has been linked to p53 activation, increased protein stability, and optimal p53 transactivation. Several p53 deacetylases have been identified including MDM2, a HDAC1-containing complex, and SIRT-2.

II Product Information

Trevigen's Anti-Mouse p53 Acetylated Lys 379 Polyclonal Antibody was raised against a peptide corresponding to amino acids 374 - 386 of the murine sequence with an acetylated lysine at position 379. The antibody is affinity purified from rabbit serum using acetylated peptide. The purified samples are then passed over a column conjugated with the unmodified peptide to remove antibody that cross reacts with non-acetylated p53. In ELISA, there is no detectable cross reaction with unmodified peptide. This antibody is ideal for use in detection of mouse acetylated p53 at Lysine 379 following immunoprecipitation of total p53 from cell lysates.

This antibody is provided in phosphate buffered saline, 50% glycerol. Store at -20°C in a manual defrost freezer.

III. Sample Preparation

For detection of acetylated p53, the method of cell harvest, lysis, and storage must maximize retention of the modified p53. Trevigen recommends the use of protease inhibitors for cell lysis and specific inhibitors of acetylase when appropriate. This protocol is provided as a guide only.

A. Pretreatment options

a) Induction of p53

In some cells, p53 can be upregulated without DNA damage which may be useful to help generate a control. For example, treat cells with 20 µM calpain inhibitor I (ALLN) for 2 - 4 hours prior to harvest.

b) Inhibition of deacetylase

To maximize the amount of acetylated p53, the use of inhibitors of deacetylases are recommended. For example, treat cells with 5 µM trichostatin A for 2 -4 hours prior to harvest.

B. Lysis Buffer

Prepare fresh lysis buffer on ice from stock solutions.

An example of an appropriate lysis buffer is:

- 50 mM Tris-Cl, pH 7.5
- 5 mM EDTA
- 150 mM sodium chloride
- 1% Triton X-100
- 50 mM sodium fluoride
- 10 mM sodium pyrophosphate
- 25 mM β -glycerophosphate
- 1 mM sodium ortho vanadate
- 1 mM sodium molybdate
- 10 μ g/ml aprotinin
- 10 μ g/ml leupeptin
- 5 μ g/ml pepstatin
- 0.5 mM PMSF

C. Lysis of suspension cells

Prechill all tubes and solutions on ice before use.

1. Harvest cells by centrifugation at 250 x g, for 10 minutes at 4 °C.
2. Wash twice in ice cold 1 X PBS.
3. Add appropriate amount of ice cold lysis buffer to give 10⁷ cells/ml.
4. Vortex cell lysate or sonicate briefly.
5. Centrifuge at 12,000 x g for 20 minutes at 4°C.
6. Decant supernatant to a fresh tube chilled on ice.
7. Aliquot appropriate volume of sample for immunoprecipitation.
8. Store unused aliquots at -80 °C and avoid freeze/thaws.

D. Lysis of adherent cells

Prechill all tubes and solutions on ice before use.

1. Place plate on ice, rinse twice with ice-cold 1 X PBS.
2. Tilt plate and remove as much of the buffer as possible.
3. Add appropriate amount of lysis buffer to give 10⁷ cells/ml.
4. Scrape cells and transfer solubilized material to prechilled tube.
5. Vortex cell lysate or sonicate briefly.
6. Centrifuge at 12,000 x g for 20 minutes at 4°C.
7. Decant supernatant to a fresh tube chilled on ice.
8. Aliquot appropriate volume of sample for immunoprecipitation.
9. Store unused aliquots at -80 °C and avoid freeze/thaws.

IV Immunoprecipitation/Western Blotting

For immunoprecipitation (IP) studies you will require an anti-p53 antibody conjugated to agarose beads. These antibodies are available from a variety of sources. Please check that the antibody you select for IP recognizes the species you are using and that it does not bind at or close to the modification site you are studying. It has been noted that acetylation of Lys 382 inhibits recognition by PAb421. Typically 5 μ g of agarose-conjugated anti-p53 antibody is used per mg of total protein in your cell lysate, however, follow the manufacturer's specific instructions for IP.

Immunoprecipitation of total p53 protein

Following incubation for 2 - 4 hours at 4 °C with rotation or rocking, wash beads at least 5 times with ice cold lysis buffer. Remove excess buffer. Add 50 μ l of 2X SDS PAGE loading buffer and boil for 3 minutes. Perform SDS PAGE and Western transfer. As a guide for mini-gels load immunoprecipitated material from approximately 300 μ g of cell lysate per lane. Depending upon the expression level of the p53 protein, more or less total protein may be needed.

Western Blotting of Immunoprecipitated p53

1. Block membrane with 5% non-fat milk in 10 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.05% Tween-20 (TBST) for 30 minutes at room temperature.
2. Incubate for 1 hour at room temperature in Anti-Mouse p53 Acetylated Lys 379 Antibody (Cat# 2370-PC-050) in TBST. Typical dilutions for Western blotting are 1:500 but empirical testing will be required for optimal results. The antibody solution may be stored at 4°C for up to 1 week and may be reused.
3. Wash 4 times for 15 minutes each wash in TBST.
4. Develop using anti-rabbit secondary antibody conjugated to peroxidase followed by chemiluminescent detection.

V. References

Appella, E. and C.W. Anderson. 2001. Post-translational modifications and activation of p53 by genotoxic stresses. *Eur. J. Biochem.* **268**:2764-2772.

Langley, E., M. Pearson, M. Faretta, U-M. Bauer, R.A. Frye, S. Minucci, P. G. Pelicco, and T. Kouzarides. 2002 Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. *The EMBO J.* **21**:2382-2396.

VI. Related Products Available from Trevigen

Anti-Human p53 Acetylated Lys 382	Cat# 2371-PC-050
Anti-Mouse/Human p53 Acetylated Lys 317	Cat# 2372-PC-050
Anti-Human Wip-1 Monoclonal Antibody	Cat# 2380-PC-050
10X PBS, pH 7.4 (6 x 500 ml)	Cat# 4870-500-6