



## Anti-mouse Acetylated p53 Antibody

### ORDERING INFORMATION

**Catalog Number:** 2370-PC-050

**Size:** 50 µg

**Formulation:** PBS and 50% glycerol

**Storage:** -20° C

**Specificity:** Acetylated p53 (K379)

**Immunogen:** Peptide corresponding to amino acids 374 - 386 of the mouse p53 sequence

**Ig class:** Rabbit IgG

**Application:** Western blot of immunoprecipitated p53

### 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.

### Preparation

This antibody was raised against a peptide corresponding to amino acids 374 - 386 of the mouse p53 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.

### Specificity

In ELISA, there is no detectable cross-reactivity with unmodified peptide. This antibody is ideal for use in detection of mouse p53 acetylated at Lysine 379 following immunoprecipitation of total p53 from cell lysates.

### Formulation

This antibody is provided in phosphate buffered saline, 50% glycerol.

### Storage

Store at ≤ -20° C in a manual defrost freezer for a minimum of 1 year. **Avoid repeated freeze-thaw cycles.**

### Sample Preparation

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

#### A. Pretreatment options

1. 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 the cells with 20 µM calpain inhibitor I (ALLN) for 2 - 4 hours prior to harvesting.
2. Inhibition of deacetylase  
To maximize the amount of acetylated p53, the use of inhibitors of deacetylases are recommended. For example, treat the cells with 5 µM trichostatin A for 2 - 4 hours prior to harvesting.

#### 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 iodide
- 1% Triton X-100
- 50 mM sodium fluoride
- 10 mM sodium pyrophosphate
- 25 mM β-glycerophosphate
- 1 mM sodium orthovanadate
- 1 mM sodium molybdate
- 10 µg/mL aprotinin
- 10 µg/mL leupeptin
- 5 µg/mL pepstatin
- 0.5 mM PMSF

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### C. Lysis of suspension cells

Prechill all tubes and solutions on ice before use.

1. Harvest the cells by centrifuging at 250 x g for 10 minutes at 2 - 8° C.
2. Wash twice in cold 1X PBS.
3. Add an appropriate amount of cold lysis buffer to give 1 x 10<sup>7</sup> cells/mL.
4. Vortex the cell lysates or sonicate briefly.
5. Centrifuge at 12,000 x g for 20 minutes at 2 - 8° C.
6. Decant the supernates to a fresh tube chilled on ice.
7. Aliquot an appropriate volume of sample for immunoprecipitation.
8. Store unused aliquots at -80° C and avoid repeated freeze-thaw cycles.

### D. Lysis of adherent cells

Prechill all tubes and solutions on ice before use.

1. Place the plate on ice and rinse twice with cold 1X PBS.
2. Tilt the plate and remove as much of the buffer as possible.
3. Add an appropriate amount of lysis buffer to give 1 x 10<sup>7</sup> cells/mL.
4. Scrape the cells and transfer the solubilized material to a prechilled tube.
5. Vortex the cell lysates or sonicate briefly.
6. Centrifuge at 12,000 x g for 20 minutes at 2 - 8° C.
7. Decant the supernates to a fresh tube chilled on ice.
8. Aliquot an appropriate volume of sample for immunoprecipitation.
9. Store the unused aliquots at -80° C and avoid repeated freeze-thaw cycles.

## Application

### Western Blotting of Immunoprecipitated p53

#### Immunoprecipitation of total p53 protein

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

Following incubation for 2 - 4 hours at 2 - 8° C with rotation or rocking, wash the beads at least 5 times with cold lysis buffer. Remove excess buffer. Add 50 µL 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 µg of cell lysate per lane. Depending upon the expression level of the p53 protein, more or less total protein may be needed.

#### Western blot of immunoprecipitated p53 protein

1. Block the membrane with 5% non-fat milk in 10 mM Tris-Cl, pH 7.5, 150 mM NaCl, 0.05% Tween<sup>®</sup> 20 (TBST) for 30 minutes at room temperature.
2. Incubate for 1 hour at room temperature with anti-mouse p53 Acetylated Lys 379 Antibody (Cat. # 2370-PC-050) in TBST. Typical dilutions for Western blotting are 1:50, but empirical testing will be required for optimal results. The antibody solution may be stored at 2 - 8° C for up to 1 week and may be reused.
3. Wash 4 times for 15 minutes each in TBST.
4. Develop using an anti-rabbit secondary antibody conjugated to peroxidase, followed by chemiluminescent detection.

## References

1. Appella, E. and C.W. Anderson (2001) *Eur. J. Biochem.* **268**:2764.
2. Langley, E. *et al.* (2002) *EMBO J.* **21**:2382.

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