

## p53 Antibody Sampler Kit

Cat# ASK07

**Background:** The human p53 tumor suppressor gene encodes a 393 amino acid phosphoprotein that exhibits sequence-specific DNA binding and directly interacts with various cellular and viral proteins (reviewed in 1). p53 is the most commonly mutated gene in human cancer, with the majority of the mutations being amino acid substitutions (2). The normal function of p53 is to effect cell cycle arrest at the G1 and G2 checkpoints in response to DNA damage (3-5). This checkpoint function is executed by accumulation of p53 followed by induction of the GADD45, WAF1 and MDM2 genes (5-7). The current model of p53 function postulates that p53 senses DNA damage and arrests the cell cycle in either the G1 or G2 phases to allow DNA repair to take place. If repair is not successful, p53 initiates programmed cell death, thus preventing the propagation of genetic defects to successive generations of cells.

### antibodies

antibody	Cat#	clone	isotype	epitope (of human p53)	species reactivity
p53 (Ab-1)	OP03	PAb421	mouse IgG <sub>2a</sub> κ	amino acids 371-380	H,M,R others
p53 (Ab-2)	OP09	PAb1801	mouse IgG <sub>1</sub> κ	amino acids 46-55	H (not M,R)
p53 (Ab-3)	OP29	PAb240	mouse IgG <sub>1</sub> κ	amino acids 213-217	H,M,R others
p53 (Ab-4)	OP32	PAb246	mouse IgG <sub>1</sub> κ	conformational	M (not H)
p53 (Ab-5)	OP33	PAb1620	mouse IgG <sub>2a</sub> κ	conformational	H,M,R
p53 (Ab-6)	OP43	DO-1	mouse IgG <sub>2a</sub> κ	amino acids 21-25	H (not M,R)

legend: H=human, M=mouse, R=rat

**How Supplied:** 20 µg of each antibody in 200 µL (100 µg/mL) of 0.05 M sodium phosphate buffer containing 0.1% sodium azide and 0.2% gelatin.

**Storage:** Store at 4°C. **Do not freeze.** If stored under proper conditions product guaranteed until expiration date stated.

**Comments:** Cat# OP03, Cat# OP09 and Cat# OP43 are pantropic (detect both mutant and wild-type p53). Cat# OP32 and Cat# OP33 detect only wild-type p53. Cat# OP29 detects only mutant p53 under nondenaturing conditions (immunoprecipitation, immunofluorescence and frozen sections) but is pantropic under denaturing conditions (western blotting and paraffin sections). Wild-type p53 has a short half-life and is present in low amounts; thus, it can be difficult to detect. For immunoprecipitations, increasing the amount of sample to be immunoprecipitated and applied to the gel may help visualize wild-type p53, and short incubation times with <sup>35</sup>S-Met (≤ 1 hr) will help reduce background. To increase sensitivity in a western blot, load more sample (i.e. 150 µg total cell protein per lane) and/or increase the concentration of primary antibody. To minimize extra bands in a western blot, reduce the concentration of both primary and secondary antibodies.

Breast carcinoma can be used as a positive control (40% will be positive) for frozen and paraffin sections. p53 (Ab-3) will give light staining of paraffin sections after pretreating with 6M urea for 10 min. at 95°C (8). Ab-4 and Ab-5 (9) have been used in frozen sections but are *not* recommended for western blotting. All antibodies in this kit are too dilute to be used in gel shift assays.

## Applications:

Western Blotting	antibody	amount	detection	positive control	negative control
	p53 (Ab-1)	10 µg/mL	chemi. or color	A431 cells	SK-OV-3 cells
	p53 (Ab-2)	2.5 µg/mL	chemi. or color	A431 cells	SK-OV-3 cells
	p53 (Ab-3)	5 µg/mL	chemi. or color	A431 cells	SK-OV-3 cells
	p53 (Ab-6)	0.1 µg/mL	chemi. or color	A431 cells	SK-OV-3 cells

Immuno-precipitation	antibody	amount	p53 detected	positive control	negative control
	p53 (Ab-1)	1 µg per sample	wt and mutant	A431 cells	SK-OV-3 cells
	p53 (Ab-2)	1 µg per sample	wt and mutant	A431 cells	SK-OV-3 cells
	p53 (Ab-3)	1 µg per sample	mutant only	A431 cells	SK-OV-3 cells
	p53 (Ab-4)	1 µg per sample	wt only	SV-T2 cells	SK-OV-3 cells
	p53 (Ab-5)	1 µg per sample	wt only	Hs27 cells	SK-OV-3 cells
	p53 (Ab-6)	1 µg per sample	wt and mutant	A431 cells	SK-OV-3 cells

Paraffin Sections	antibody	amount	pretreatment	positive control	negative control
	p53 (Ab-2)	5 µg/mL	pepsin or heat	see comments	normal skin
	p53 (Ab-5)	5 µg/mL	heat	see comments	normal skin
	p53 (Ab-6)	1 µg/mL	pepsin or heat	see comments	normal skin

Frozen Sections	antibody	amount	p53 detected	positive control	negative control
	p53 (Ab-1)	10 µg/mL	wt & mutant	see comments	normal skin
	p53 (Ab-2)	5 µg/mL	wt & mutant	see comments	normal skin
	p53 (Ab-3)	10 µg/mL	mutant only	see comments	normal skin
	p53 (Ab-6)	1 µg/mL	wt & mutant	see comments	normal skin

Immuno-fluorescence	antibody	amount	p53 detected	positive control	negative control
	p53 (Ab-1)	1-20 µg/mL	wt and mutant	A431 cells	SK-OV-3 cells
	p53 (Ab-3)	1-20 µg/mL	mutant only	A431 cells	SK-OV-3 cells
	p53 (Ab-4)	1-20 µg/mL	wt only	SV-T2 cells	SK-OV-3 cells
	p53 (Ab-5)	1-20 µg/mL	wt only	Hs27 cells	SK-OV-3 cells
	p53 (Ab-6)	1 µg/mL	wt and mutant	A431 cells	SK-OV-3 cells

See Comments section (next page) for additional applications information.

## References:

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