

# Product Information

sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103 USA

Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757

email: techservice@sial.com sigma-aldrich.com

## Monoclonal Anti-p63 Clone 4A4

produced in mouse, purified immunoglobulin

Catalog Number **P3737**

### Product Description

Monoclonal Anti-p63 (mouse IgG2a isotype) is derived from the 4A4 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with a recombinant N-terminal portion (amino acids 1-205) of mouse  $\Delta$ Np63 isoform.<sup>1</sup> The isotype is determined using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. The antibody is purified from culture supernatant of hybridoma cells grown in a bioreactor.

Monoclonal Anti-p63 recognizes an epitope within amino acids 1-205 of mouse  $\Delta$ Np63 isoform. This epitope is common to all mouse p63 isoforms.<sup>1</sup> The antibody may be used in ELISA,<sup>1</sup> immunoblotting,<sup>1,4</sup> (3 bands at approx. 50, 60, and 70 kDa in HaCaT cells, and a variety of approx. 44, 60,<sup>3</sup> 80,<sup>1-3</sup> and 90-95<sup>1</sup> kDa, in various preparations), immunohistochemistry<sup>1-4</sup> (frozen sections and Bouin's fluid-,<sup>2</sup> 10% buffered formalin-,<sup>2,4</sup> or 4% paraformaldehyde<sup>2</sup>-fixed paraffin-embedded<sup>1-4</sup> sections), immunocytochemistry and immunoprecipitation. Reactivity has been observed with human,<sup>1,3</sup> rat and mouse<sup>1-4</sup> p63.

Human cancer is a multistep process, in which there is an accumulation of genetic damage to key regulatory genes, known as oncogenes and tumor-suppressor genes. The p53 tumor suppressor gene is one of the most frequently mutated genes in human cancers. p53 is a sequence-specific transcription factor. Under conditions of genotoxic stress, it plays a critical role in activating the expression of genes involved in cell cycle arrest or apoptosis. Two other genes, referred to as p63 and p73, have been found to encode proteins that share a significant amino acid identity with p53 in the transactivation domain, the DNA binding domain, and the oligomerization domain.<sup>5-7</sup> Like p53, these proteins can recognize canonical p53 DNA-binding sites. When overproduced they activate p53-responsive target genes and induce apoptosis. p63, together with p73, transactivates p53-regulated promoters and induces apoptosis via some mechanisms that are utilized by p53, but also by others completely distinct from those engaged by p53.<sup>8</sup> In contrast to p53, p63 and p73 are rarely mutated in human cancer.<sup>9</sup> In addition, these genes, unlike p53, undergo complex alternative splicing, which at least in the case of p63, yields proteins with widely divergent biological properties.

The p63 gene expresses at least six major transcripts, with predicted molecular masses ranging from 44 kDa to 72 kDa, derived from alternative splicing events. The central domain of all p63 variants is highly homologous to the DNA-binding domains of p53 and p73, suggesting that at least some p63 isoforms function as transcriptional activators.<sup>1</sup> The encoded proteins have two different N-termini (TA\*/TA and  $\Delta$ N) and three different C-termini ( $\alpha$ ,  $\beta$  and  $\gamma$ ). Three of the encoded protein isoforms, TAp63 $\alpha$ , TAp63 $\beta$  and TAp63 $\gamma$ , contain the transactivation (TA) domain and are able to transactivate p53 reporter genes and induce apoptosis. In contrast, the other three isoforms,  $\Delta$ Np63 $\alpha$ ,  $\Delta$ Np63 $\beta$  and  $\Delta$ Np63 $\gamma$ , lack the acidic, N-terminal TA domain and act as dominant-negative factors to suppress transactivation by both p53 and TAp63 isoforms.<sup>1,3</sup>

p63 is highly expressed in the basal or progenitor layers of many epithelial tissues,<sup>1-3</sup> and the major p63 variants of these basal cells lack the N-terminal transactivation domain.<sup>1</sup> p63 is critical for maintaining the progenitor-cell populations that are necessary to sustain epithelial development and morphogenesis.<sup>2,3</sup> Indeed, p63 germline inactivation in the mouse results in agenesis of organs such as skin appendages, breast and prostate.<sup>2,3,10</sup> Prostate basal cells, but not secretory or neuroendocrine cells, express p63. In addition, prostate basal cells in culture predominantly express the  $\Delta$ Np63 $\alpha$  isoform. In contrast, p63 protein is not detected in human prostate adenocarcinomas.<sup>3</sup> Thus, p63 immunohistochemistry may be a valuable tool in the differential diagnosis of benign versus malignant prostatic lesions.<sup>3</sup>

Monoclonal antibody reacting specifically with p63 is a useful tool for the study of the molecular mechanisms by which p63 can exert dominant-negative and gain-of-function, involved in development and regulation.

### Reagent

Supplied as a solution in 0.01 M PBS, pH 7.4, containing 1% BSA and 15 mM sodium azide.

Antibody concentration: ~2 mg/mL

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### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

### Product Profile

Immunoblotting: a working concentration of 1-2 µg/mL is recommended using a whole extract of cultured human keratinocyte HaCaT cells.

**Note:** In order to obtain the best results in various techniques and preparations, we recommend determining optimal working concentration by titration.

### References

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KAA,LPG,PHC 02/08-1