

## Product Information

### ANTI DP-1

Developed in Rabbit,  
IgG Fraction of Antiserum

Product Number **D 6562**

#### Product Description

Anti DP-1 is developed in rabbit using a synthetic human DP-1 N-terminal peptide conjugated to maleimide activated KLH as immunogen. The peptide corresponds to amino acid residues 9-27 of the DP-1 molecule with a C-terminal added cysteine.

Anti-DP1 reacts specifically with the DP-1 transcription regulator. The antibody may be used for immunoblotting (~50 kD) using whole cell lysates of DP-1 transfected cells, or *in-vitro* translated DP-1 protein. Staining of the DP-1 band is specifically inhibited with the immunizing DP-1 peptide by immunoblotting. The antibody may also be used for immunofluorescent staining and immunoprecipitation of DP-1 protein in transfected cells.

The E2F family of transcription factors is important for cell cycle control. The E2F transcription factors are heterodimeric molecules composed of two distinct proteins termed E2F and DP. The E2F component of this protein complex is encoded by a family of genes consisting of at least 6 members (E2F-1 to -6). The DP family contains three proteins (DP -1 to -3), which heterodimerize interchangeably with any of the E2F proteins.

E2F/DP target genes include genes important for DNA replication (e.g. DHFR, ORC1, MCM), cyclin genes (A and E), proto-oncogene (myc, myb) E2F genes and the Rb family genes (Rb, p107).

The E2F/DP transcription factors are tightly regulated by multiple regulatory mechanisms. Besides being regulated by the combination of the two components, the E2F/DP complex is negatively regulated by binding to one or more members of the retinoblastoma (Rb) pocket protein family: pRb, p107 and p130. Complexes of unphosphorylated pRb and E2F/DP act as transcriptional repressors that contribute to the Rb-

dependent G1 arrest. Once a pocket protein is phosphorylated by CDKs, the E2F/DP/Pocket protein complex dissociates, resulting in free, transcriptionally active E2F/DP heterodimer. Another level of regulation is the synthesis and degradation of the proteins: certain E2F species are actively degraded by the ubiquitin-proteasome pathway, and their synthesis is cell-cycle regulated.<sup>2, 3</sup>

The E2F proteins can be grouped into 3 subfamilies based upon structural homology and functional characteristics. The first subfamily consists of E2F-1, -2, and -3 which bind to and are regulated only by pRb. This group of proteins all contain a dedicated cyclin binding sequence N-terminal to the DNA binding domain, which mediates the binding of cyclin A/cdk2. The second family consists of E2F-4 and -5, which bind mainly to p107 and p130 and sometime to pRb in case of E2F-4<sup>1</sup>. E2F-6 (also known as EMA) seems to belong to another subfamily because it does not bind any of the pRb family members (it lacks a pocket protein-binding domain) and its overexpression seems to delay exit from S-phase rather than induce S-phase.<sup>4</sup> DP1 is a cytosolic protein that migrates to the nucleus once it forms stable association with E2F1, E2F2 or E2F3, as has been demonstrated in transfection experiments<sup>5</sup>.

Antibodies to DP1 are a useful tool for the detection, localization and for following the intracellular trafficking of DP-1 protein in cells.

#### Reagents

Anti DP-1 is supplied as an IgG fraction of antiserum in 0.01M phosphate buffered saline, pH 7.4, containing 15mM sodium azide.

#### Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous 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 is not recommended. 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**

A minimum working dilution of 1:3,000 is determined by immunoblotting using a whole extract of transfected cells expressing recombinant human DP-1 or *in-vitro* translated DP-1 protein.

A minimum working dilution of 1:100 is determined by immunofluorescent staining of transfected cells expressing recombinant human DP-1.

A minimum working dilution of 1:80 is sufficient to precipitate DP-1 protein in transfected cells expressing recombinant human DP-1.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilution by titration test.

**References**

1. Moberg, K., et al., Mol. Cell Biol., **16**, 1436 (1996).
2. Hateboer, G. et al., Genes Dev., **10**, 2960 (1996).
3. Hofman, F., et al., Genes Dev., **10**, 2949 (1996).
4. Cartwright, P., et al., Oncogene, **6**, 611 (1998).

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