

## Product Information

### ANTI- E2F1

Developed in Rabbit, Affinity Isolated Antibody

Product Number **E 9026**

#### Product Description

Anti-E2F1 is developed in rabbit using a synthetic human E2F1 C-terminal peptide conjugated to KLH with glutaraldehyde as immunogen. The peptide corresponds to amino acid residues 310-329 of the E2F1 molecule. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-E2F1 reacts specifically with the E2F1 transcription regulator protein (recombinant). The antibody may be used for immunoblotting (~50 kDa) and immunoprecipitation techniques using whole cell extracts of E2F1 transfected cells, or *in vitro* translated E2F1 proteins. Staining of the E2F1 band by immunoblotting is specifically inhibited with the immunizing E2F1 peptide.

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*) 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 that bind to and are regulated only by pRb. All members of this group of proteins contain a dedicated cyclin binding sequence N-terminal to the DNA binding domain that mediates the binding of cyclin A/cdk2. The second family consists of E2F-4 and -5, which bind mainly to p107 and p130 and sometimes to pRb in the 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> E2F1 synthesis is cell cycle regulated in cells emerging from G0. In growth arrested cells, E2F1 is undetectable but its levels rise during late G1. E2F1 is accumulated in the nuclei. The E2F1 promoter contains E2F DNA binding sites and is cell cycle regulated. Antibodies to E2F1 are a useful tool for the detection and localization of E2F1 protein in cells.<sup>5</sup>

#### Reagents

Anti-E2F1 is supplied as an affinity isolated antibody in 10 mM phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.

Protein concentration is approximately 0.8 mg/ml.

#### 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 concentration of 2.5 µg/ml is determined by immunoblotting using a whole extract of transfected cells expressing recombinant human E2F1, or *in vitro* translated E2F1 protein.

A minimum working dilution of 10 µg of antibody will immunoprecipitate E2F1 from extracts of transfected cells expressing recombinant human E2F1.

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions 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. Hofmann, F., et al., Genes Dev., **10**, 2949 (1996).
4. Cartwright, P., et al., Oncogene, **6**, 611 (1998).
5. Magae, J., et al., Oncogene, **18**, 593 (1999).

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