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Certificate of Analysis

p27 complex, activated

(Recombinant protein expressed in Sf21 insect cells)
Item # 23-024, 23-024-K, 23-024M
Parent Lot # D11DP017N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: Complex of *N*-terminal c-Myc-tagged, recombinant human p27 full length, 6His-tagged, recombinant human Cyclin E1 full length and untagged, recombinant human CDK2 full length, co-expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography. Phosphorylated *in vitro* using CDK2/Cyclin E1.

Purity 94.6% by SDS-PAGE and Coomassie blue staining. p27 MW = 23kDa, CDK2 MW = 34kDa, Cyclin E1 MW = 48kDa

Formulation: 0.444mg/ml of enzyme in 25mM MOPS pH7.5, 5mM MgCl2, 0.01% Tween 20, 1mM ATP. Frozen solution.

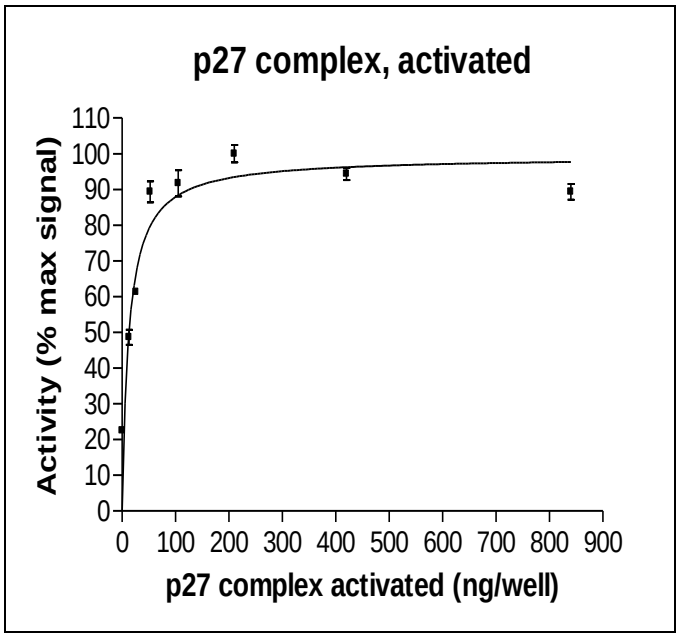
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

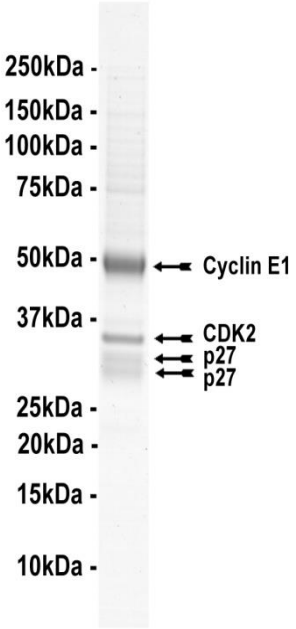
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Assay: This enzyme was titrated in a ubiquitination assay and the results normalised against the maximum signal.



Protein Identity: Confirmed identity as p27, CDK2 and Cyclin E1 by mass spectrometry.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of p27 complex, activated.

* note: p27 migrates as a doublet at ~27–31kDa.

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Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH3, active (Item # 23-022)**
3. **$SCF^{Skp2/Cks1}$ complex, active (Item # 23-023)**
4. **1x Reaction Buffer**
5. **Biotinylated-Ubiquitin**
6. **Stop Solution**

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$).

p27 complex, activated is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$, 10µM ATP, 10nM UBE1, 500nM UbcH3, 5ug/ml $SCF^{Skp2/Cks1}$ complex and 2µM biotinylated-ubiquitin. The reaction is initiated with the addition of biotinylated-ubiquitin. After 30 minutes at room temperature the reaction is terminated by the addition of 25mM MOPS pH7.5 containing 125mM EDTA, 150mM NaCl and 0.05% Tween 20. Reaction products are separated by capture onto a microplate coated with anti-c-Myc antibody and washing with PBS containing 0.05% Tween 20. Ubiquitination of p27 complex is measured by detection of bound ubiquitin via electrochemiluminescence.

p27 Complex Information

Protein human p27, human CDK2, human Cyclin E1

Accession number GenBank BC001971 p27, GenBank NM_001798 CDK2, GenBank NM_001238 Cyclin E1

Alternative Names $p27^{kip1}$, Kip1, Cyclin-dependent kinase inhibitor 1B

Key Facts p27 is an important regulator of cell cycle progression, involved in G1 arrest. It is a potent inhibitor of cyclin E1- and cyclin A-CDK2 complexes and a positive regulator of cyclin D-dependent kinases such as CDK4. The degradation of this protein, which is triggered by its CDK-dependent phosphorylation and subsequent ubiquitination by SCF complexes, is required for the cellular transition from quiescence to the proliferative state. Decreased levels of p27 are found in various epithelial tumours originating from lung, breast, colon, ovary, oesophagus, thyroid and prostate. Defects in the gene encoding p27 (CDKN1B) are the cause of multiple endocrine neoplasia type 4 (MEN4) an inherited cancer of the thyroid.

Related Products Item # 23-021 UBE1, active, Item # 23-022 UbcH3, active, Item # 23-023 $SCF^{Skp2/Cks1}$ complex, active

Selected References

Besson A. *et al*. Discovery of an Oncogenic Activity in $p27^{Kip1}$ that Causes Stem Cell Expansion and a Multiple Tumor Phenotype. Genes Dev., 21*:* 1731-1746, 2007

Grimmler M. *et al.* Cdk-Inhibitory Activity and Stability of $p27^{Kip1}$ are Directly Regulated by Oncogenic Tyrosine Kinases. Cell, 128*:* 269-280, 2007

Lee J. and Kim S. S. The Function of $p27^{Kip1}$ During Tumor Development. Exp Mol Med. 41: 765–771, 2009

Ungermannova D. *et al.* Ubiquitination of $p27^{Kip1}$ Requires Physical Interaction with Cyclin E and Probable Phosphate Recognition by SKP2. J. Biol. Chem., 280: 30301–30309, 2005

Pellegata N. S. *et al.* Germ-line Mutations in $p27^{Kip1}$ Cause a Multiple Endocrine Neoplasia Syndrome in Rats and Humans. PNAS, *103:* 15558-15563, 2006

Reviewed and approved by site quality representative.