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Certificate of Analysis

p53

(Recombinant protein expressed in Sf21 insect cells)
Item # 23-034, 23-034-K, 23-034M
Parent Lot # 2468533

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: *N*-terminal c-Myc, 6His-tagged, recombinant human p53 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 90.8% by SDS-PAGE and Coomassie blue staining. MW = 49kDa.

Formulation: 1.320mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

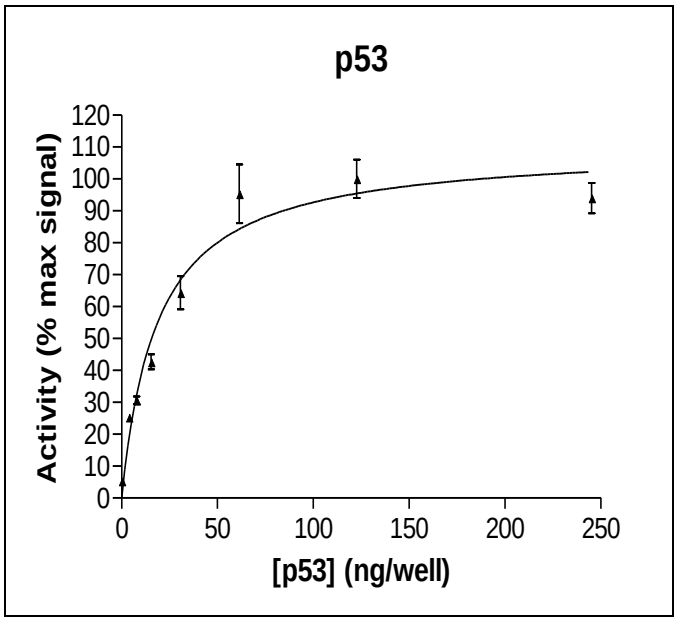
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

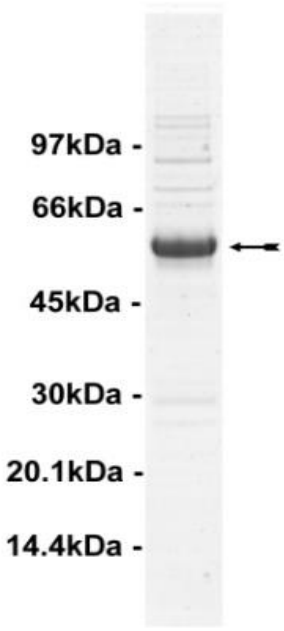
Quality Control Testing

Assay: This protein was titrated in a ubiquitination assay. The results were normalised against the maximum signal.



Protein Identity: Confirmed identity as p53 by mass spectrometry.

SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of p53.



Eurofins Pharma Discovery Services UK Limited
Gemini Crescent
Dundee Technology Park
DUNDEE
DD2 1SW United Kingdom

T | +44 (0)1382 561600
F | +44 (0)1382 561601
www.eurofins.com/pharmadiscovery

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Assay Protocol

Reagents:

1. **UBE1 (Item # 23-021)**
2. **UbcH4 (Item # 23-025)**
3. **MDM2/CK1δ, active (Item # 23-032)**
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 $MgCl_2$).

p53 is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$, 10µM ATP, 10nM UBE1, 500nM UbcH4, 1.25ug/ml MDM2/CK1δ 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 p53 is measured by detection of bound ubiquitin via electrochemiluminescence.

p53 Information

Protein human p53

Accession number GenBank BC003596

Alternative Names Cellular tumour antigen p53, Antigen NY-CO-13, Phosphoprotein p53, Tumour suppressor p53, TP53

Key Facts p53 is expressed in a wide variety of tissues and plays a major role in preventing tumour development**.** It is involved in cell cycle regulation, functioning as a trans-activator to negatively regulate cell division by controlling a set of genes required for this process. It acts as a tumour suppressor in many tumour types by stimulating either apoptosis or growth arrest depending on the physiological circumstances and the cell type. Defects in p53 expression have been implicated in several diseases including; choroid plexus papilloma, lung cancer, head/neck squamous cell carcinomas, oesophageal squamous cell carcinoma, Li-Fraumeni syndrome, and hereditary adrenocortical carcinoma.

Related Products Item # 23-021 UBE1, active, Item # 23-025 UbcH4, active, Item # 23-035 UbcH5c, active, Item # 23-032 MDM2/CK1δ, active

Selected References

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Hock A. and Vousden K.H. Regulation of the p53 Pathway by Ubiquitin and Related Proteins. Int J Biochem Cell Biol. *42*: 1618-1621, 2010

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Petitjean A. *et al.,* Impact of Mutant p53 Functional properties on TP53 Mutation Patterns and Tumor Phenotype: Lessons From Recent Developments in the IARC TP53 Database. Hum Mutat. *28*: 622-629, 2007

Kruse J-P. and Gu W. Modes of p53 Regulation. Cell, *137:* 609-622, 2009

Reviewed and approved by site quality representative.