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Certificate of Analysis

p53 (expressed in *E coli*)

Item # 14-952, 14-952-K, 14-952M
Parent Lot # WAD0330

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 cMyc and GST-tagged, recombinant, human p53 full length expressed in *E coli*. Purified using glutathione agarose and gel filtration. Purity 82% by SDS-PAGE and Coomassie blue staining. MW = 72kDa.

Formulation: 0.34mg/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.

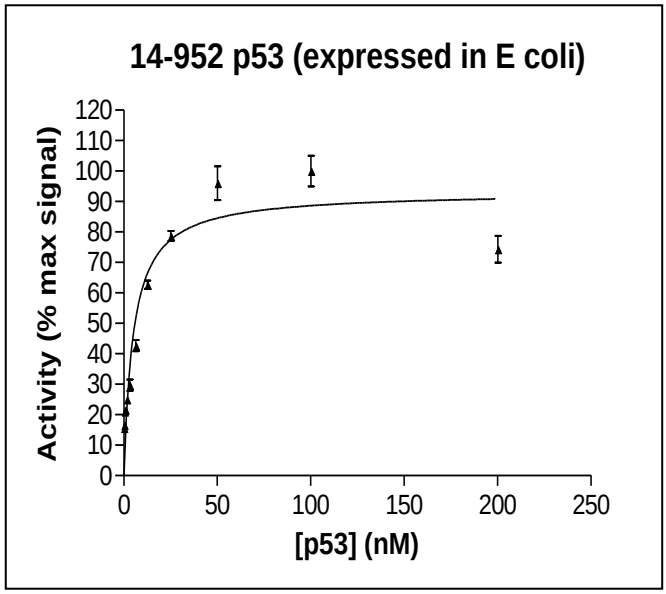
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

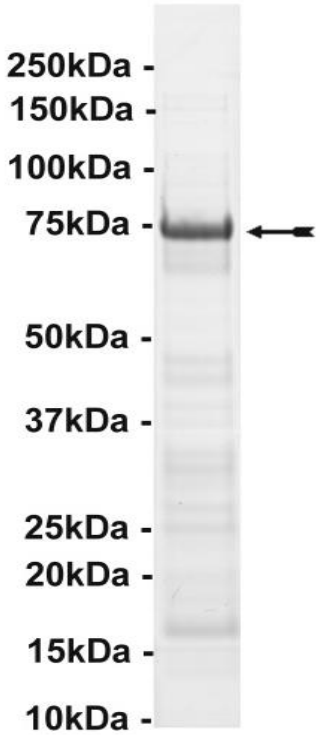
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 0–200nM of this lot of p53 was tested as a substrate capable of phosphorylation by DNA-PK, active (Eurofins cat# 14-950) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed identity as p53 with the translated sequence listed on page three



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/pharamdiscovery

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Phosphorylation of p53 by DNA-PK, active

Reagents:

1. **1 x Reaction Buffer:** 25mM HEPES pH8.0, 0.01% Brij-35, 1% Glycerol.
2. **Dilution Buffer:** 25mM HEPES pH8.0, 0.01% Brij-35, 1% Glycerol, 5mM DTT, 1mg/ml BSA
3. **ATP Solution (4x):** 400µM ATP, 40mM Magnesium Acetate.
4. **p53 (expressed in *E.coli*):** Dilute with 25mM HEPES pH8.0, 0.01% Brij-35, 1% Glycerol. Use 0–200nM per assay point.
5. **DNA-PK, active (Eurofins cat # 14-950):** Dilute with 25mM HEPES pH8.0, 0.01% Brij-35, 1% Glycerol, 5mM DTT, 1mg/ml BSA. Use 0.5ng per assay well.
6. **Stop Solution:** 12.5mM HEPES pH8.0, 0.005% Brij-35, 0.5% Glycerol, 250mM EDTA.
7. **Detection Mix:** 50mM HEPES pH7.0, 150mM NaCl, 267mM KF, 0.1% sodium cholate, 0.01% Tween 20, 0.0125% sodium azide, anti-phospho-p53 (Ser15)-K (CisBio 61P08KAE) 0.42ng/well, and anti-GST-d2 (CisBio 61GSTDLA) 25ng/well.

Suggested Assay Procedure (384 well plate format):

The volumes detailed below are suitable for a 384-well plate (e.g. Corning Costar 3573) using a 20µL reaction volume (30µL stopped volume).

Assay Procedure

1. Add 10µl of 1 x reaction buffer per assay to wells.
2. Add 2.5µl of (0–200nM) p53.
3. Add 2.5µl (0.5ng) **DNA-PK, active**.
4. Add 5µl of ATP mixture to initiate the reaction.
5. Incubate for 30 minutes at room temperature.
6. Stop the reaction by adding 5µl of the 12.5mM HEPES pH8.0, 0.005% Brij-35, 0.5% Glycerol, 250mM EDTA.
7. Add 5µL Detection Mix
8. It is recommended that the plate is sealed to minimize reduction in reaction volume. It is recommended that the plate is read after an overnight incubation following the termination of the reaction and addition of the Detection Mix.
9. Measure HTRF ratio on an appropriate microplate reader according to the following parameters:

Excitation	330 - 380nm
Emission	665 - 667.5nm and 620 - 635nm
Counting Delay	50µsec
Counting window	(integration time) 400µsec

Refer to your instrument manufacturer for further guidance on measurement parameters recommended for HTRF.

Calculation:

HTRF Ratio is calculated as follows:

$$HTRF\ Ratio = \left(\frac{Emission\ at\ 665nm}{Emission\ at\ 620nm} \right) \times 10000\,.$$

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p53 Sequence Information

Protein human p53

Tags *N*-terminal cMyc and *N*-terminal GST

Native sequence M240 of the recombinant protein is equivalent to M1 of human p53

Accession number GenBank BC003596.1

Recombinant p53 amino acid sequence:

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  1 MEQKLISEED LSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL
 61 EFPNLPYYID GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA
121 YSKDFETLKV DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL
181 DAFPKLVCFK KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LVPRGSKEFM
241 EEPQSDPSVE PPLSQETFSD LWKLLPENNV LSPLPSQAMD DLMLSPDDIE QWFTEDPGPD
301 EAPRMPEAAP RVAPAPAAPT PAAPAPAPSW PLSSSVPSQK TYQGSYGFRL GFLHSGTAKS
361 VTCTYSPALN KMFCQLAKTC PVQLWVDSTP PPGTRVRAMA IYKQSQHMTE VVRRCPHHER
421 CSDSDGLAPP QHLIRVEGNL RVEYLDDRNT FRHSVVVPYE PPEVGSDCTT IHYNYMCNSS
481 CMGGMNRRPI LTIITLEDSS GNLLGRNSFE VRVCACAGRD RRTEEENLRK KGEPHHELPP
541 GSTKRALPNN TSSSPQPKKK PLDGEYFTLQ IRGRERFEMF RELNEALELK DAQAGKEPGG
601 SRAHSSHLKS KKGQSTSRHK KLMFKTEGPD SD
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Recombinant p53 nucleotide sequence:

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   1 atggaacaaa aattgatttc ggaagaagac ttgtccccta tactaggtta ttggaaaatt
  61 aagggccttg tgcaacccac tcgacttctt ttggaatatc ttgaagaaaa atatgaagag
 121 catttgtatg agcgcgatga aggtgataaa tggcgaaaca aaaagtttga attgggtttg
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 301 gagatttcaa tgcttgaagg agcggttttg gatattagat acggtgtttc gagaattgca
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 421 aaaatgttcg aagatcgttt atgtcataaa acatatttaa atggtgatca tgtaacccat
 481 cctgacttca tgttgtatga cgctcttgat gttgttttat acatggaccc aatgtgcctg
 541 gatgcgttcc caaaattagt ttgttttaaa aaacgtattg aagctatccc acaaattgat
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1801 agcagggctc actccagcca cctgaagtcc aaaaagggtc agtctacctc ccgccataaa
1861 aaactcatgt tcaagacaga agggcctgac tcagactga
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Reviewed and approved by site quality representative.