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Certificate of Analysis

Kit

(Recombinant protein expressed in Sf21 insect cells)
Item # 23-043, 23-043-K, 23-043M
Parent Lot # D11HP002N

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, GST-tagged, recombinant human Kit amino acids 544-end, expressed by baculovirus in Sf21 insect cells. Purified using glutathione sepharose.

Purity 81% by SDS-PAGE and Coomassie blue staining. MW = 78kDa.

Formulation: 0.295mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

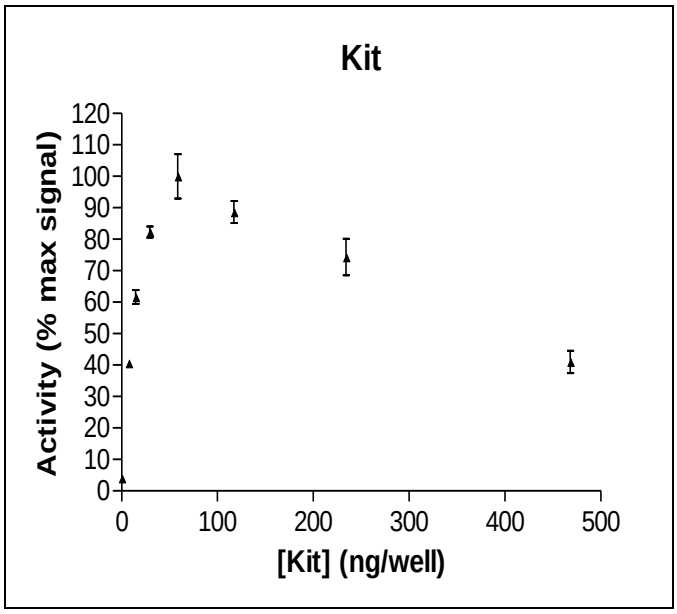
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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

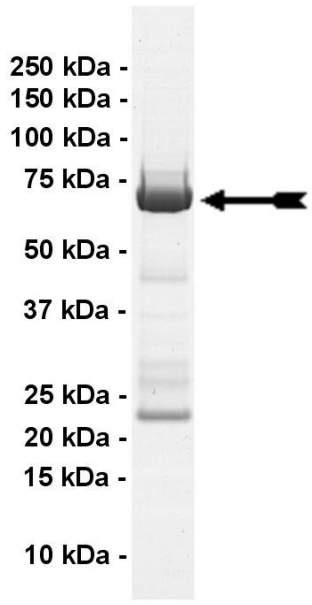
Quality Control Testing

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



Protein Identity: Confirmed identity as Kit by mass spectrometry.

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



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, active (Item # 23-021)**
2. **UbcH4, active (Item # 23-025)**
3. **c-Cbl, active (Item # 23-041)**
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 $MgCl_2$).

Kit is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$, 10µM ATP, 10nM UBE1, 100nM UbcH4, 5µg/ml c-Cbl, 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 Kit is measured by detection of bound ubiquitin via electrochemiluminescence.

Kit Information

Protein human Kit

Accession number GenBank X06182

Alternative Names Mast/stem cell growth factor receptor, Proto-oncogene c-Kit, Tyrosine-protein kinase Kit, CD_antigen=CD117

Key Facts Kit belongs to the family of type III receptor tyrosine kinases. It mediates the cellular response to stem cell factor and plays a major role in survival, expansion and differentiation of haematopoietic progenitor cells and reproductive stem cells. The activation of Kit is tightly regulated and uncontrolled Kit activity contributes to the growth of diverse human tumours. Gain-of-function mutations have been described in a number of malignancies, including acute myeloid leukaemia, small cell lung cancer, gastrointestinal stromal tumours and testicular cancer. Negative regulation of Kit signalling has been shown to occur mainly through ubiquitin-mediated internalization and degradation of the receptor.

Related Products Item # 23-021 UBE1, active, Item # 23-025 UbcH4, active, Item # 23-041 c-Cbl,active

Selected References

Yarden Y. *et al.,* Human Proto-Oncogene c-kit: a New Cell Surface Receptor Tyrosine Kinase for an Unidentified Ligand. EMBO J. *6*: 3341-3351, 1987

Ashman L. K. The Biology of Stem Cell Factor and its Receptor c-kit. Int J Biochem Cell Biol. *31*: 1037-1051, 1999

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Longley B. J. *et al.,* Classes of c-Kit Activating Mutations: Proposed Mechanisms of Action and Implications for Disease Classification and Therapy. Leuk Res. *25*: 571-576, 2001

Masson K. *et al.,* Direct Binding of Cbl to Tyr^{568} and Tyr^{936} of the Stem Cell Factor Receptor/c-Kit is Required for Ligand-Induced Ubiquitination, Internalization and Degradation. Biochem J. *399*: 59-67, 2006

Reviewed and approved by site quality representative.