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Certificate of Analysis

TRAF6, active

(Recombinant E3 ligase expressed in *E.coli*)
Item # 23-059, 23-059-K, 23-059M
Parent Lot # D13EP006N

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 GST-tagged, recombinant human TRAF6 full length, expressed in *E.coli*. Purified using glutathione sepharose.
Purity 54% by SDS-PAGE and Coomassie blue staining. MW = 87kDa.

Activity (Parent lot# D13EP006N): This lot of TRAF6 is active and meets product specifications.

Formulation: 0.647mg/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.

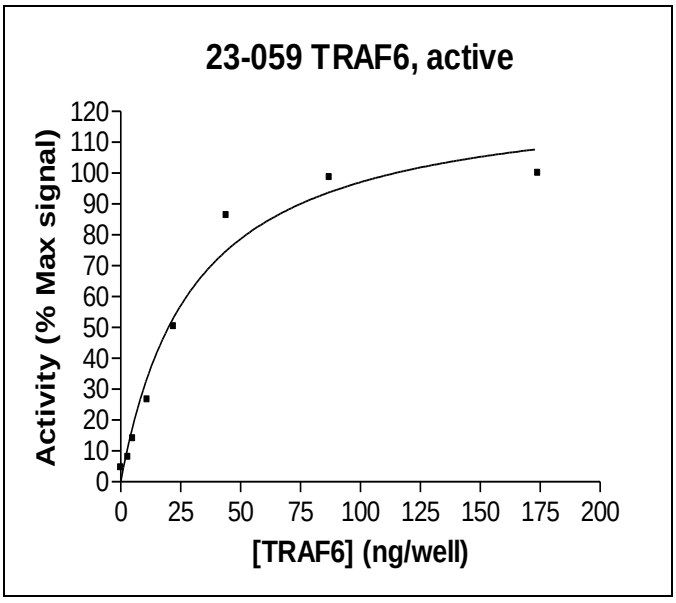
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

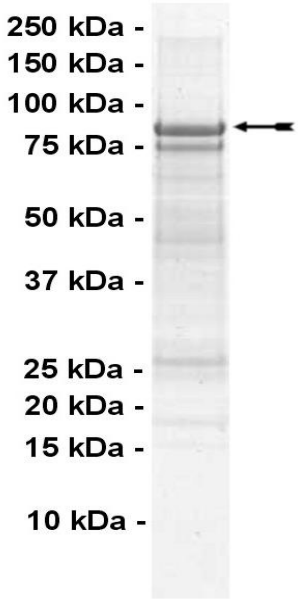
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

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



Protein Identity: Confirmed identity as TRAF6 by mass spectrometry.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of TRAF6, active.

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E3 Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH5a, active (Item # 23-029)**
3. **TRAF6, active (Item # 23-059)**
4. **IKKγ (Item # 23-060)**
5. **1x Reaction Buffer**
6. **Biotinylated-Ubiquitin**
7. **Stop Solution**

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH 7.5, 0.01% Tween 20, 5mM $MgCl_2$).

TRAF6 is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$, 10µM ATP, 10nM UBE1, 1000nM UbcH5a, 25nM IKKγ, 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. TRAF6 activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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TRAF6 Information

Protein Human TRAF6

Accession number GenBank BC031052

Alternative Names TNF receptor-associated factor 6, Interleukin-1 signal transducer, RING finger protein 85.

Key Facts TRAF6 is a ubiquitin ligase essential for the activation of NF-κB and MAP kinases in multiple signalling pathways including those emanating from the tumour-necrosis-factor receptor (TNFR) and interleukin-1/Toll-like receptors (IL-1R/TLR). TRAF6 also transduces signals from receptors such as CD40 and RANK and may be essential for the formation of functional osteoclasts. Studies indicate that amplification of the TRAF6 locus is a somatic and frequent event in several human cancer types and is a commonly amplified oncogene bridging RAS and NF-κB in lung cancer. Inhibition of TRAF6 in lung cancer cells has been shown to suppress NF-κB activation, anchorage-independent growth and tumour formation.

Related Products UBE1, active (Item # 23-021), UbcH5a, active (Item # 23-029), IKKγ (Item # 23-060)

Selected References

Sun H. *et al.*, TRAF6 upregulates expression of HIF-1α and promotes tumour angiogenesis. Cancer Res., *73*: 4950-4959, 2013.

Muto G. *et al*., TRAF6 is essential for maintenance of regulatory T cells that suppress Th2 type autoimmunity. PLoS One., *8*: 1-12, 2013

Inoue J. *et a*l., Characteristics and biological functions of TRAF6. Adv Exp Med Biol., *597*: 72-79, 2007.

Chen Z.J. Ubiquitin signaling in the NF-κB pathway. Nat Cell Biol., *7*: 758–765, 2005.

Reviewed and approved by site quality representative.