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Certificate of Analysis

Smad7

(Recombinant protein expressed in Sf21 insect cells)
Item # 23-039, 23-039-K, 23-039M
Parent Lot # D11HP006N

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 Smad7 full length, expressed by baculovirus in Sf21 insect cells. Purified using immobilized metal affinity chromatography.

Purity 87% by SDS-PAGE and Coomassie blue staining. MW = 51kDa.

Formulation: 0.585mg/ml of enzyme in 50mM Tris/HCl pH8.0, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

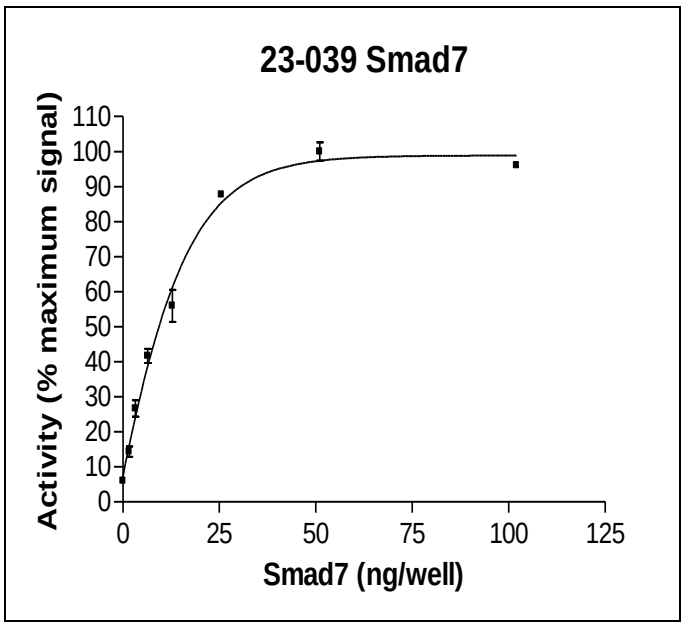
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

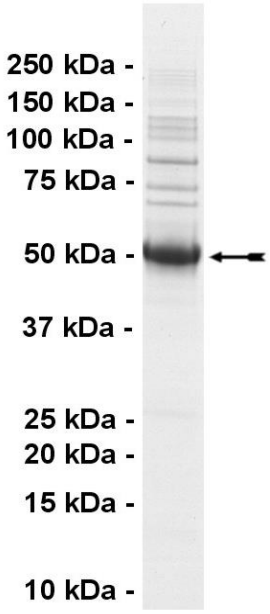
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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 Smad7 by mass spectrometry.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Smad7.

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Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH5c, active (Item # 23-035)**
3. **Smurf1, active (Item # 23-058)**
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 pH 7.5, 0.01% Tween 20, 5mM $MgCl_2$).

Smad7 is incubated with 25mM MOPS pH 7.5, 0.01% Tween 20, 5mM $MgCl_2$, 10µM ATP, 10nM UBE1, 1000nM UbcH5c, 0.05µg Smurf1, 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 pH 7.5 containing 125mM EDTA, 400mM NaCl, and 1% Triton. 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 Smad7 is measured by detection of bound ubiquitin via electrochemiluminescence.

Smad7 Information

Protein human Smad7

Accession number GenBank NM_005904

Alternative Names Mothers against decapentaplegic homologue 7, MAD homologue 7, Mothers against DPP homologue 7, Mothers against decapentaplegic homologue 8, MADH7, MADH8

Key Facts The TGF-*β* signalling cascade regulates diverse cellular processes, including cell proliferation, differentiation, apoptosis, adhesion and migration. It plays a fundamental role in a number of different physiological and pathological processes, such as embryonic development, adult homoeostasis, tumourigenesis and tissue fibrogenesis. Smad7 has been identified as a key negative regulator of TGF-*β* signalling, antagonising it through multiple mechanisms in both the cytoplasm and in the nucleus. Altered expression of Smad7 is often associated with human diseases, such as cancer, tissue fibrosis and inflammatory diseases.

Related Products Item # 23-021 UBE1, active, Item # 23-035 UbcH5c, active, Item # 23-058 Smurf1, active

Selected References

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He W. *et al*., Overexpression of Smad7 Results in Severe Pathological Alterations in Multiple Epithelial Tissues. EMBO J., *21*: 2580-2590, 2002

Park J. Fine Tuning and Cross-talking of TGF-β Signal by Inhibitory Smads. J. Biochem Mol Biol. *38*: 9-16, 2005

Yan X. and Chen Y-G. Smad7: Not Only a Regulator, but also a Cross-Talk Mediator of TGF-*β* Signalling. Biochem. J. *434*: 1–10, 2011

Broderick P. A Genome-Wide Association Study Shows that Common Alleles of SMAD7 Influence Colorectal Cancer Risk. Nat Genet. *39*:1315-1317, 2007

Reviewed and approved by site quality representative.