

## Certificate of Analysis

### Smurf1, active

(Recombinant E3 ligase expressed in *E coli*)

Item # 23-058, 23-058-K, 23-058M

Parent Lot # D12EP007N

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 Smurf1 full length, expressed in *E coli*. Purified using glutathione sepharose.

Purity 59% by SDS-PAGE and Coomassie blue staining. MW = 110kDa.

#### Activity (Parent lot# D12EP007N):

This lot of Smurf1, active is active and meets product specifications.

**Formulation:** 0.186mg/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.

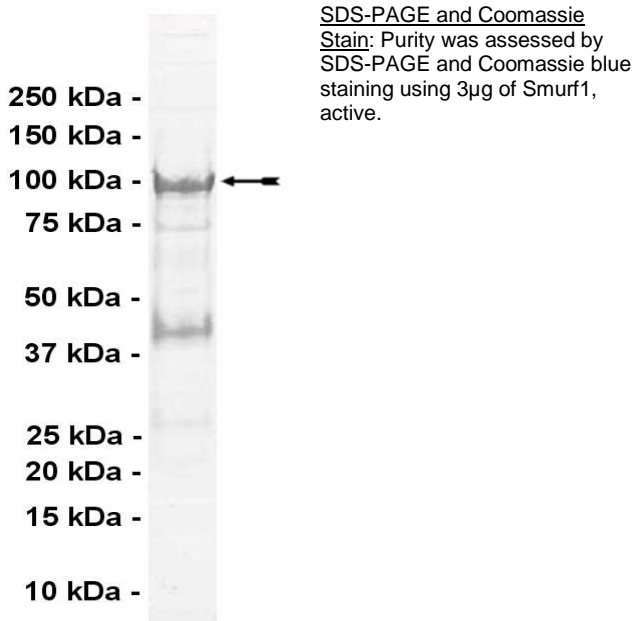
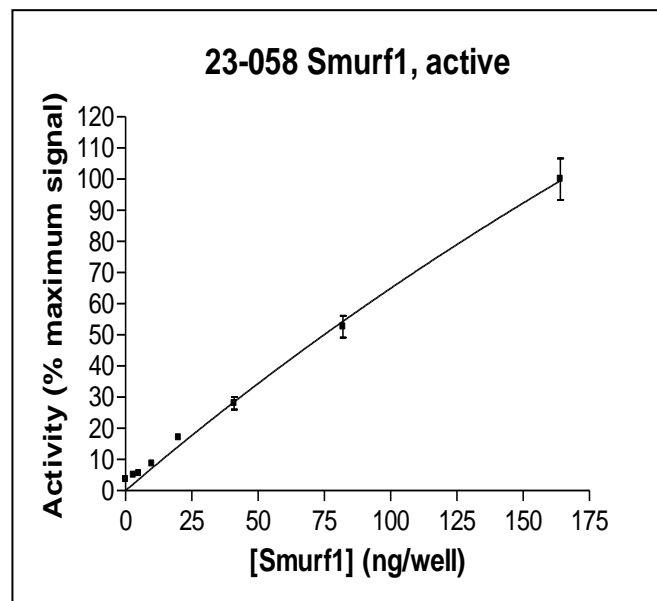
**Handling Recommendations:** Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

**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 Smurf1 by mass spectrometry.



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### E3 Assay Protocol

#### Reagents:

- |                                   |                           |
|-----------------------------------|---------------------------|
| 1. UBE1, active (Item # 23-021)   | 5. 1x Reaction Buffer     |
| 2. Ubch5c, active (Item # 23-035) | 6. Biotinylated-Ubiquitin |
| 3. Smurf1, active (Item # 23-058) | 7. Stop Solution          |
| 4. Smad7 (Item # 23-039)          |                           |

#### Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH 7.5, 0.01% Tween 20, 5mM MgCl<sub>2</sub>).

Smurf1 is incubated with 25mM MOPS pH 7.5, 0.01% Tween 20, 5mM MgCl<sub>2</sub>, 10μM ATP, 10nM UBE1, 1000nM Ubch5c, 50nM Smad7, 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. Smurf1 activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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### Smurf1 Information

<b><u>Protein</u></b>	human Smurf1
<b><u>Accession number</u></b>	GenBank NM_181349
<b><u>Alternative Names</u></b>	SMAD ubiquitination regulatory factor 1, SMAD-specific E3 ubiquitin-protein ligase 1
<b><u>Key Facts</u></b>	<p>Smurf1 is a member of the Nedd4-like family of E3 ligases. It contains an amino terminal C2 domain and 2 WW domains that are responsible for cellular localization and substrate recognition, and a catalytic carboxyl terminal HECT domain.</p> <p>Smurf1 was originally identified as a negative regulator of the TGF-<math>\beta</math>/BMP (bone morphogenetic proteins) signalling pathway by degrading SMAD proteins and TGF-<math>\beta</math>/BMP receptors. To date, over a dozen substrates have been identified, including Runx2, MEKK2, RhoA, Par6, JunB, Talin head, Prickle1, TRAF family proteins, and STAT1. This suggests a pleiotropic role for Smurf1 in various cellular functions such as bone homeostasis, embryonic development, cell movement and polarity control, and immune response. Aberrant expression of Smurfs has been shown to occur in several types of cancers, including breast, oesophageal, pancreatic and renal cell carcinomas.</p>
<b><u>Related Products</u></b>	Item # 23-021 UBE1, active, Item # 23-035 Ubch5c, active, Item # 23-039 Smad7

### **Selected References**

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Li S. *et al.*, Ubiquitin Ligase Smurf1 Targets TRAF Family Proteins for Ubiquitination and Degradation. *Mol Cell Biochem*. 338: 11-17, 2010

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Reviewed and approved by site quality representative.

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