PRODUCT DATA SHEET
Affimer (HS1-A1) to Human SUMO1

Overview

Product Name	Anti-Human SUMO1 Affimer (HS1-A1)
Catalogue Code	AVA00024
Description	Affimer (HS1-A1) to Human SUMO1
Clone ID	HS1-A1
Tested Applications	Co-Immunoprecipitation ITC Western Blot
Tags	C-term 6His
Conjugate	None

Properties

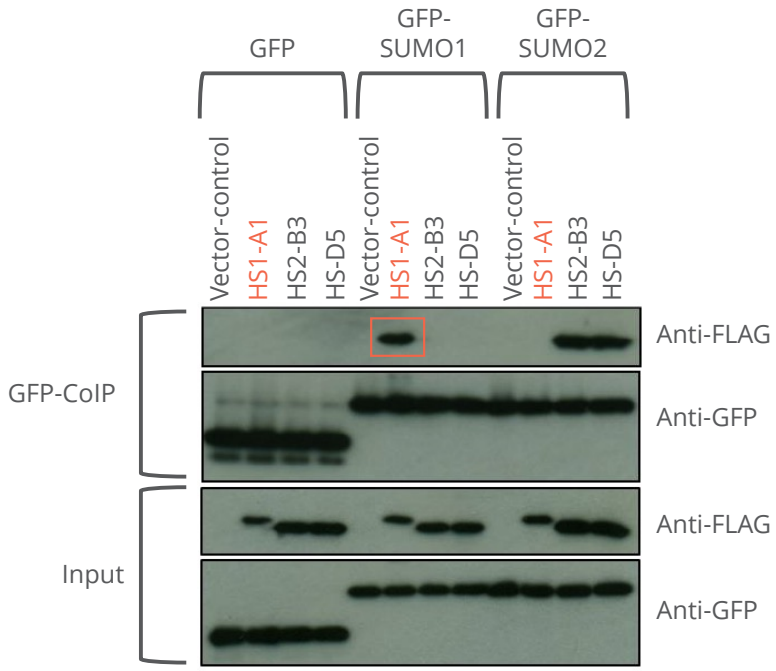
Form	Liquid
Storage Instructions	For short term use, store at 4°C. We recommend aliquoting and storing at -20°C long term. Affimers are generally unaffected by 3-4 freeze/thaw cycles.
Buffer	100mM Sodium Phosphate, 75mM Sodium Chloride, 0.02% Sodium Azide, pH 7.4
Purity	>95%
Purification Method	IMAC
Clonality	Monoclonal

Target

Target	Human SUMO1
Affimer Reactivity	Human
Target Uniprot ID	P63165
Target Function	SUMO proteins are ubiquitin-like proteins that can be covalently attached to proteins as a monomer or a lysine-linked polymer. Covalent attachment via an isopeptide bond to its substrates requires prior activation by the E1 complex SAE1-SAE2 and linkage to the E2 enzyme UBE2I, and can be promoted by E3 ligases such as PIAS1-4, RANBP2 or CBX4. Polymerisation is mediated by RNF4. This post-translational modification on lysine residues of proteins plays a crucial role in a number of cellular processes such as nuclear transport, DNA replication and repair, mitosis and signal transduction. It is involved in targeting RANGAP1 to the nuclear pore complex protein RANBP2. SUMO1 chains are also susceptible to polyubiquitination which functions as a signal for proteasomal degradation of modified proteins. SUMO proteins interact with SAE2, PML, RANGAP1, p53, MDM2, JUN and HIF1A, May also regulate a network of genes involved in palate development.
Research Area	Cell Signalling / UPS

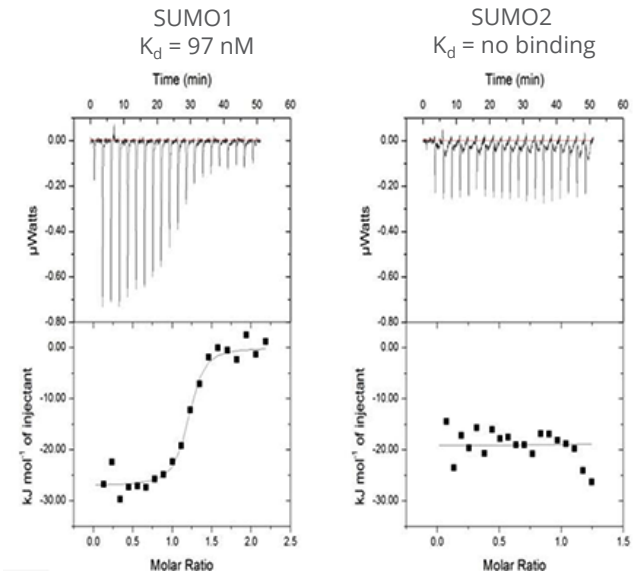
Applications

Co-Immunoprecipitation



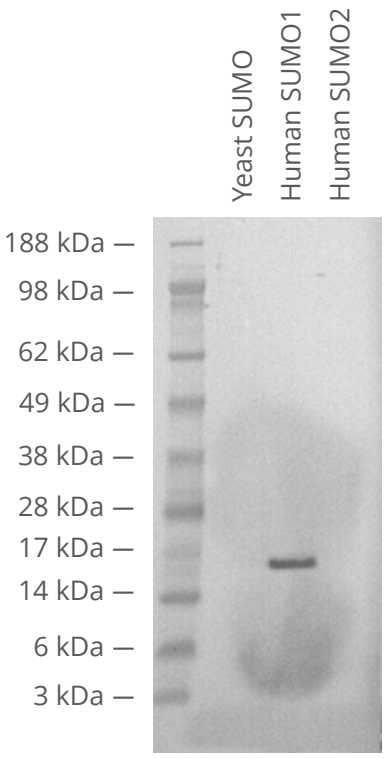
HEK293T cells were (co)-transfected with plasmids bearing the target (GFP, GFP-SUMO1 or GFP-SUMO2) and Affimer (with FLAG tags) for 48 h. Cells were washed in PBS and proteins extracted in 1 ml lysis buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% NP-40 and 1x protease inhibitor cocktail (Roche) for 15 min on ice and clarified by centrifugation at 12,000 xg for 10 min, 4°C. Lysates were incubated with GFP-Trap (Chromotek) for co-immunoprecipitation of GFP-labelled species. Immunoprecipitated proteins were eluted in Laemmli buffer and subject to immunoblot analysis. As marked by the red box, Affimer (HS1-A1) co-precipitates in the presence of GFP-SUMO1 but not GFP-SUMO2.

ITC



Clone HS1-A1 was shown to specific for Human SUMO1 (Kd = 97nM) but has no interaction with Human SUMO2 by isothermal titration calorimetry.

Western Blot



SDS-PAGE & Blotting: Protein samples (100 ng per well) were separated on a Bolt 4-12% Bis-Tris Plus gel (Life Tech) and blotted onto Nitrocellulose using an iBlot2 (Life Tech).

Blocking: The membrane was blocked using 1 x TBS + 3% skimmed milk powder (1 h, RToC, 400 rpm)

Wash 1: 3 x 5ml of 1xTBS-T (0.05% Tween-20)

Affimer Incubation: 5µg/ml in 1 x TBS + 3% skimmed milk powder (16 h, 4oC, 400 rpm)

Wash 2: 3 x 5ml of 1xTBS-T (0.05% Tween-20)

Detection: Rabbit anti-6xHis HRP conjugate, 1/5,000 dilution in 1 x TBS + 3% skimmed milk powder (1 h, RToC, 400 rpm)

Wash 3: 3 x 5ml of 1xTBS-T (0.05% Tween-20)

Substrate: ECL (Amersham)