Datasheet for ABIN4368250

anti-Arginylation (N-Term) antibody

2 Validations

Go to Product page

Overview

Quantity:	2 x 25 µg
Target:	Arginylation
Binding Specificity:	N-Term
Reactivity:	Please inquire
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	Un-conjugated
Application:	Dot Blot (DB)

Product Details

Immunogen:	KLH-conjugated synthetic peptide: H-RDHKHANQHMSVC-NH2
Specificity:	N-terminal arginylation
Purification:	Affinity purified serum. Antibodies are purified using substactive purification method.

Target Details

Target:	Arginylation
Target Type:	Chemical
Background:	Arginylation is a post-translational modification of an existing peptide chain by addition of an extra arginine. This modification changes primary sequence of protein as well as it's surface change. It is mediated by arginyltransferase ATE1. Arginylation plays an essential role in multiple physiological pathways, for example, in vivo arginylation constitutes a mechanism for

Target Details

	degradation of preprocessed proteins or proteolytic fragments that bear Asp and Glu on their N-termini.

Application Details

Application Notes:	1 : 1000 (Dot)
Restrictions:	For Research Use only

Handling

Format:	Lyophilized
Reconstitution:	For reconstitution add 25 µL of sterile water to each tube.
Buffer:	PBS pH 7.4
Storage:	-20 °C
Storage Comment:	Store lyophilized/reconstituted at -20°C, once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.

Successfully validated (Western Blotting (WB))

by Leibniz-Institut für Pflanzenbiochemie (IPB), Halle

Report Number: 100047

Date: Sep 02 2016

Target:	Arginylation (N-Term)
Method validated:	Western Blotting (WB)
Positive Control:	Arg-Asp-starting recombinant protein
Negative Control:	Asp-starting recombinant protein
Notes:	ABIN4368250 specifically detects recombinant protein with the N-terminal amino acid sequence Arg-Asp in a Western blot.
Primary Antibody:	ABIN4368250
Secondary Antibody:	goat anti-rabbit IgG-HRP (Santa Cruz, sc-2004)

Protocol:	• Recombinant protein:
	  ◦ Bacterial protein expression and purification according to Naumann et al. 2016.
	  ◦ 1-15µg total protein were mixed with SDS-loading buffer and boiled for 5min.
	• Plant extracts:
	  ◦ Plant material (3 weeks old Arabidopsis) was ground with liquid nitrogen in RIPA-buffer and incubated for 15min at 4°C.
	  ◦ Debris was centrifuged at max. speed for 10min. Total protein content of the supernatant was determined.
	  ◦ 23.6µg (first experiment) or 94µg (second experiment) respectively were mixed with SDS-loading buffer and boiled for 5min.
	• Proteins were separated on a 12% denaturing SDS-PAGE gel (Carl Roth) in a Bio-Rad Tetra cell and transferred in a Bio-Rad semidry-blot apparatus for 1.5h at 1mA/cm^2 to PVDF membrane (GE Healthcare).
	• The membrane was blocked in TBST with 5% milk powder (Carl Roth) for 1h in at RT.
	• Incubation with primary antibody ABIN4368250 (antibodies-online) diluted 1:1000 in TBST with 3% milk powder overnight at 4°C.
	• Washing with TBST with 3% milk powder for 3x 5min.
	• Incubation with secondary antibody goat anti-rabbit IgG-HRP (Santa Cruz, sc-2004) diluted 1:2500 in TBST with 3% milk powder for 1h at RT.
	• Washing with TBST with 3% milk powder for 5x 5min.
	• Detection with SuperSignal West Pico Chemiluminescent Substrate (Thermo Scientific) on a standard X-ray film; exposure 30s.

Validation report #100047 for Western Blotting (WB)

Experimental Notes:	ABIN4368250 recognizes the recombinant protein with N-terminal Arg-Asp shown in the image. In case of a different recombinant protein (not shown) the antibody did not only recognize the protein with the N-terminal sequence Arg-Asp but also with Asn or Asp at the amino-terminus.

Image for Validation report #100047

Validation image no. 1 for anti-Arginylation (N-Term) antibody (ABIN4368250)

Detection of detect a bacterially expressed recombinant protein with ABIN4368250, either with the N-terminal amino acid sequences Arg1-Asp2 in three independent preparations (lanes 1, 2, and 3; upper panel) or an N-terminal Asp1. ABIN4368250 only recognizes the protein with the amino-terminal sequence Arg-Asp. For each preparation, 20µg of total protein were loaded onto the SDS-PAGE gel. The lower panel shows a Coomassie stain loading control.

Validation report #100046 for Protein Array (PAr)

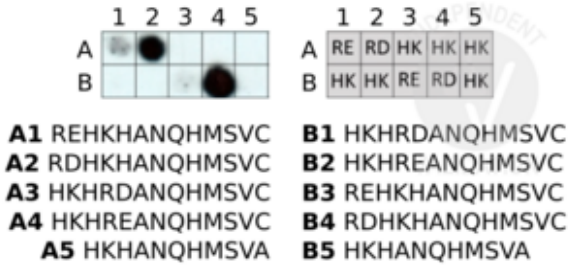
Successfully validated (Protein Array (PAr))

by Leibniz-Institut für Pflanzenbiochemie (IPB), Halle

Report Number: 100046

Date: Aug 23 2016

Target:	Arginylation (N-Term)
Method validated:	Protein Array (PAr)
Positive Control:	N-terminally arginylated synthetic peptides REHKHANQHMSVC, RDELPESIDWRKKGAV
Negative Control:	un-arginylated synthetic peptides HKHRDANQHMSVC, HKHREANQHMSVC, HKHANQHMSVA
Notes:	The antibody specifically recognizes peptides with the N-terminal amino acid sequence RD and to a lesser extent peptides with the N-terminal sequence RE.
Primary Antibody:	ABIN4368250
Secondary Antibody:	goat anti-rabbit IgG-HRP (Santa Cruz, sc-2004)
Protocol:	• Peptide array synthesis according to Yim et al. 2015. • Block membrane with TBST with 7.5% milk powder overnight at 4°C. • Incubation with primary N-terminal arginylation antibody (antibodies-online, ABIN4368250) diluted 1:1000 in TBST with 3% milk powder for 1h at RT. • Washing with TBST for 3x 10min. • Transfer proteins onto PVDF membrane in a semi-dry for 30min at 0.8mA/cm^2. • Block the membrane with TBST with 4% milk powder ON at 4°C. • Incubation with secondary antibody goat anti-rabbit IgG-HRP (Santa Cruz, sc-2004) diluted 1:2500 in TBST with 3% milk powder for 1h at RT. • Wash membrane 5x 20min in TBST. • Chemiluminescence detection with ECL (Thermo Scientific pico kit) on a standard X-ray film; exposure 30s.



Validation image no. 1 for anti-Arginylation (N-Term) antibody (ABIN4368250)

ABIN4368250 is highly specific for the sequence of the utilized antigen, i.e. N-terminal RD (peptides A1 and B4). It shows minimal cross-reactivity with N-terminal RE (A1 and B3). No interaction with un-arginylated peptides was detected.