

Datasheet for ABIN4368846

anti-SPAG4 antibody[Go to Product page](#)**2** Validations

Overview

Quantity:	100 µL
Target:	SPAG4
Reactivity:	Human, Mouse, Rat
Host:	Rabbit
Clonality:	Polyclonal
Application:	ELISA, Western Blotting (WB), Immunohistochemistry (IHC)

Product Details

Target Details

Target:	SPAG4
Alternative Name:	SPAG4 (SPAG4 Products)
Background:	Synonyms: Sperm associated antigen 4, SUN4, CT127

Application Details

Restrictions:	For Research Use only
---------------	-----------------------

Handling

Format:	Liquid
Concentration:	1.0 mg/mL
Preservative:	Sodium azide

Handling

Precaution of Use:	WARNING: Reagents contain sodium azide. Sodium azide is very toxic if ingested or inhaled. Avoid contact with skin, eyes, or clothing. Wear eye or face protection when handling. If skin or eye contact occurs, wash with copious amounts of water. If ingested or inhaled, contact a physician immediately. Sodium azide yields toxic hydrazoic acid under acidic conditions. Dilute azide-containing compounds in running water before discarding to avoid accumulation of potentially explosive deposits in lead or copper plumbing.
Storage:	-20 °C



Successfully validated (Western Blotting (WB))

by [Johann-Friedrich-Blumenbach-Institute for Zoology and Anthropology, Department of Developmental Biology, Georg-August-University Göttingen](#)

Report Number: 101449

Date: Aug 31 2017

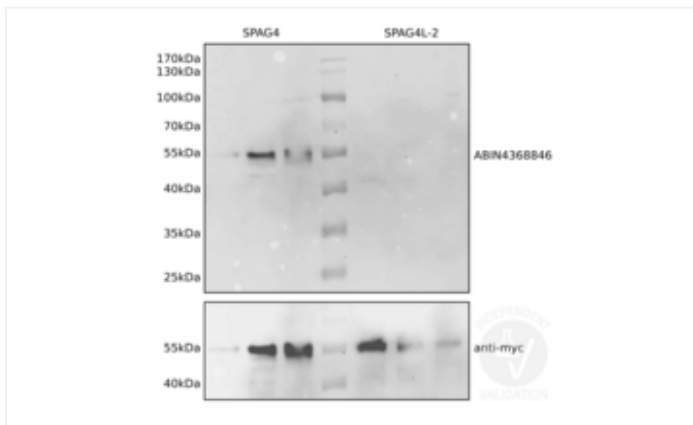
Target:	SPAG4
Lot Number:	3522
Method validated:	Western Blotting (WB)
Positive Control:	NIH/3T3 mouse embryonic fibroblast cells overexpressing HA- and myc-tagged SPAG4
Negative Control:	NIH/3T3 mouse embryonic fibroblast cells overexpressing HA- and myc-tagged SPAG4L-2
Notes:	ABIN4368846 specifically recognizes ectopically expressed SPAG4 and does not detect ectopic SPAG4L-2.
Primary Antibody:	ABIN4368846
Secondary Antibody:	anti-mouse IgG, HRP-linked (Sigma A9044, Lot 034M4761)
Protocol:	<ul style="list-style-type: none"> NIH/3T3 cells (ATCC, CRL-1658) are grown in DMEM+GlutaMAX (Gibco, 31966-021, Lot 1852045) supplemented with fetal bovine serum (Gibco 270-106) and Pen/Strep (Gibco 15140), at 37°C and 5% CO₂ to 70% confluency. Transfect cells with HA/myc-tagged SPAG4 expression plasmid or HA/myc-tagged SPAG4L-2 (Frohnert et al., 2011) expression plasmid using EndofectinMax (GeneCopoeia) following the manufacturer's instructions. Grow cells for 24h. Lyse cells in SDS-sample buffer and denature total cellular lysates for 5min at 95°C. Separate cell lysates on a denaturing 10% SDS-PAGE gel (Laemmli 1970). Transfer proteins onto 0.2 µm Protran membrane (GE Healthcare, 10600004, A10043108) with a Western blotting system for 1h at 400mA (Towbin et al., 1979). Block the membrane in TBST (50mM Tris-HCl, pH7.4, 150mM NaCl, 0.2% Tween 20) containing 5% milk (blocking solution) for 60min at RT. Incubate membrane with primary rabbit anti-Sperm Associated Antigen 4 (SPAG4) antibody (antibodies-online, ABIN4368846, lot 3522) diluted 1:1000 in blocking solution ON at 4°C. Wash membrane with TBST for 30min at RT. Incubate membrane with secondary goat anti-rabbit IgG (H+L), HRP-linked (Jackson Immuno Research, 111-035-003, lot 123450) diluted 1:4000 in blocking solution for 45min at RT. Wash membrane 6x for 5 min with TBST. Reveal protein bands using Clarity Max Western ECL substrate (Bio-Rad, 1705062) and capture images via Chemidoc Imaging System (BioRad).

Validation report #101449 for Western Blotting (WB)

- Incubate membrane with primary mouse anti-myc tag antibody (clone 9E10) diluted 1:50 in blocking solution ON at 4°C.
- Wash membrane with TBST for 30min at RT.
- Incubate membrane with secondary anti-mouse IgG, HRP-linked (Sigma A9044, Lot 034M4761) diluted 1:5000 in blocking solution for 45min at RT.
- Reveal protein bands using Clarity Max Western ECL substrate (Bio-Rad, 1705062).

Experimental Notes: ABIN4368846 passes validation when used for detection of ectopically expressed proteins. However, it reacted with proteins specifically present in testicular tissue from day 7 post-partum to the adult and even in Spag4-deficient testis (Spag4-deficiency was verified by genotyping and RT-PCR on testis cDNA).

Image for Validation report #101449



Validation image no. 1 for anti-Sperm Associated Antigen 4 (SPAG4) antibody (ABIN4368846)

Different volumes of lysates from NIH/3T3 cells ectopically expressing SPAG4 (lanes 1-3) or SPAG4L-2 (lanes 5-7) were loaded and the blot membrane was incubated with ABIN4368846 (upper panel). Ectopically expressed proteins were subsequently detected using an anti-myc-tag antibody. Expected molecular masses of SPAG4 and SPAG4L-2 are approximately 50kDa.



Successfully validated (Immunocytochemistry (ICC))

by [Johann-Friedrich-Blumenbach-Institute for Zoology and Anthropology, Department of Developmental Biology, Georg-August-University Göttingen](#)

Report Number: 101455

Date: Aug 31 2017

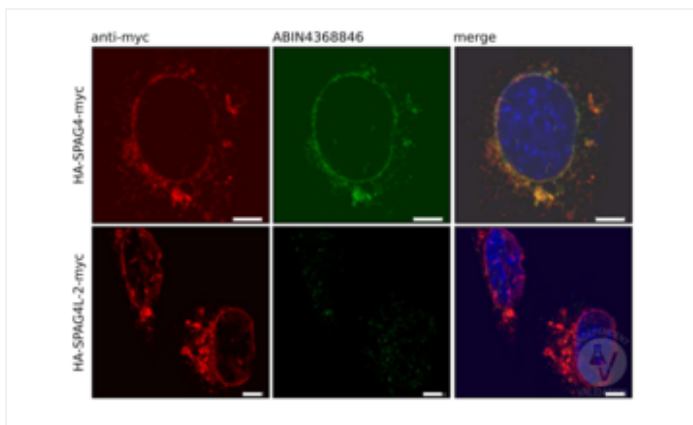
Target:	SPAG4
Lot Number:	3522
Method validated:	Immunocytochemistry (ICC)
Positive Control:	NIH/3T3 mouse embryonic fibroblast cells overexpressing HA- and myc-tagged SPAG4
Negative Control:	NIH/3T3 mouse embryonic fibroblast cells overexpressing HA- and myc-tagged SPAG4L-2
Notes:	The SPAG4 antibody ABIN4368846 specifically labels ectopically expressed Spag4 in cultured NIH/3T3 cells in ICC.
Primary Antibody:	ABIN4368846
Secondary Antibody:	goat anti-rabbit IgG DyLight488 (Thermo Scientific, 35553, lot KA130088)
Protocol:	<ul style="list-style-type: none"> NIH/3T3 cells (ATCC, CRL-1658) are grown in DMEM+GlutaMAX (Gibco, 31966-021, Lot 1852045) supplemented with fetal bovine serum (Gibco 270-106) and Pen/Strep (Gibco 15140), at 37°C and 5% CO₂ to 70% confluency. Transfect cells with HA/myc-tagged SPAG4 expression plasmid or HA/myc-tagged SPAG4L-2 (Frohnert et al., 2011) expression plasmid using EndofectinMax (GeneCopoeia) following the manufacturer's instructions. Grow cells for 24h. Fix cells in 3.7% paraformaldehyde (in PBS) for 15min at 4°C followed by incubation in 0.3% Triton X-100 for 10min at 4°C. Block cells in PBS containing 1% bovine serum albumin and 0.5% Tween-20 (PBT) for 1h at room temperature. Incubate cells with primary <ul style="list-style-type: none"> rabbit anti-Sperm Associated Antigen 4 (SPAG4) antibody (antibodies-online, ABIN4368846, lot 3522) diluted 1:100 in PBS ON at 4° and mouse anti-myc tag antibody clone (9E10) diluted 1:50 in PBS ON at 4°C. Wash cells with TBST (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% Tween 20) for 15min at RT. Incubate cells with secondary <ul style="list-style-type: none"> goat anti-rabbit IgG DyLight488 (Thermo Scientific, 35553, lot KA130088) and goat anti-mouse IgG (H+L) Alexa Fluor 555 (Invitrogen, A21422, lot 948498) diluted 1:2000 in PBS for 1h at 37°C.

- Counterstain DNA with DAPI (4',6-Diamidino-2-phenylindole; Sigma D-9542).
- Image acquisition on Zeiss LSM 510 confocal microscope and processing using Adobe Photoshop 5.0.

Experimental Notes:

- An anti-myc antibody (red) detected both myc-tagged proteins, but anti the SPAG4 antibody ABIN4368846 (green) exclusively detected SPAG4 without any cross-reaction to SPAG4L-2. ABIN4368846 detected SPAG4 at the nuclear membrane, as expected.
- However, on mouse testicular cells ABIN4368846 decorated the manchette in spermatids (as was expected) but gave a much more intense signal on cells, that do not express Spag4 ([Pasch et al., 2015](#))

Image for Validation report #101455



Validation image no. 1 for anti-Sperm Associated Antigen 4 (SPAG4) antibody (ABIN4368846)

NIH/3T3 cells expressing myc-tagged Spag4 (upper row) or Spag4L-2 (lower row) were incubated with an anti-myc tag antibody (left, red) or anti-SPAG4 antibody (middle, green). The pictures on the right show the red and green channels merged with DAPI counterstain (blue).