antibodies .- online.com







anti-SUN5 antibody





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| ()\/\ | rview |
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Application Notes:

| Quantity: | 100 μL |
|---------------------|--|
| Target: | SUN5 |
| Reactivity: | Human, Mouse, Rat |
| Host: | Rabbit |
| Clonality: | Polyclonal |
| Application: | Western Blotting (WB) |
| | |
| Product Details | |
| Isotype: | IgG |
| Characteristics: | Expected molecular weight of target: 379 kDa |
| Purification: | Affinity purification |
| | |
| Target Details | |
| Target: | SUN5 |
| Alternative Name: | SPAG4L (SUN5 Products) |
| Gene ID: | 140732 |
| UniProt: | Q8TC36 |
| | |
| Application Details | |
| | |

WB 1:500-1:2000

Application Details

Restrictions: For Research Use only

Handling

| Format: | Liquid |
|--------------------|--|
| Concentration: | 1 mg/mL |
| Buffer: | PBS with 0.02 % sodium azide, 50 % glycerol, pH 7.4 |
| Preservative: | Sodium azide |
| Precaution of Use: | This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only. |
| Handling Advice: | Avoid repeated freeze/thaw cycles. |
| Storage: | -20 °C |
| Storage Comment: | Store at -20°C. |





Successfully validated (Immunocytochemistry (ICC))

by Johann-Friedrich-Blumenbach-Institute for Zoology and Anthropology, Department of Developmental Biology, Georg-August-University Göttingen

Report Number: 102344

Date: Dec 19 2017

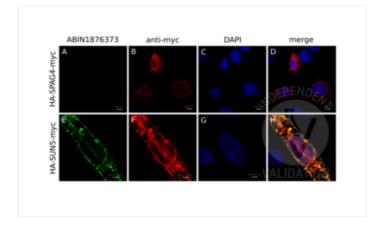
| Target: | SUN5 |
|---------------------|--|
| Method validated: | Immunocytochemistry (ICC) |
| Positive Control: | NIH/3T3 mouse embryonic fibroblast cells overexpressing HA- and myc-tagged SPAG4L-2/SUN5 |
| Negative Control: | NIH/3T3 mouse embryonic fibroblast cells overexpressing HA- and myc-tagged SPAG4 |
| Notes: | Passed. ABIN1876373 is specific for SPAG4L-2/SUN5 and did not detect SPAG4 when ectopically expressed. Unspecific binding to other cellular proteins is nearly absent. |
| Primary Antibody: | ABIN1876373 |
| Secondary Antibody: | goat anti-rabbit IgG DyLight488 (Thermo Scientific, 35553, lot KA130088) |
| Protocol: | 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/SUN5 (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 rabbit anti-SUN5 antibody (antibodies-online, ABIN1876373) diluted 1:100 in PBS ON at 4° and mouse anti-myc tag antibody clone (9E10) diluted 1:100 in PBS ON at 4°C. Wash cells with TBST (50mM Tris-HCl, pH 7.4, 150mM NaCl, 0.1% Tween 20) for 15min at RT. Incubate cells with secondary goat anti-rabbit IgG DyLight488 (Thermo Scientific, 35553, lot KA130088) diluted 1:1000 in PBS for 1h at 37°C and goat anti-mouse IgG (H+L) Alexa Fluor 555 (Invitrogen, A21422, lot 948498) diluted 1:1000 |



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.

Image for Validation report #102344



Validation image no. 1 for anti-Sad1 and UNC84 Domain Containing 5 (SUN5) antibody (ABIN1876373)

Specificity of ABIN1876373. Cells were transfected either with SPAG4 (upper row) or SPAG4L-2/SUN5 (lower row), both tagged with HA and myc. Anti-myc antibody (B, F; red) detected both proteins, but ABIN1876373 (A, E; green) exclusively detected SPAG4L-2/SUN5 without any crossreaction to SPAG4. The antibody detected SPAG4L-2/SUN5 at the nuclear membrane, as expected. The pictures on the right (D, H) show the red and green channels merged with DAPI counterstain (blue).