Datasheet for ABIN2443910

anti-HA-Tag antibody

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Overview

Quantity:	100 µg
Target:	HA-Tag
Reactivity:	Please inquire
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This HA-Tag antibody is un-conjugated
Application:	Western Blotting (WB), ELISA, Immunoprecipitation (IP), Immunofluorescence (IF), Immunohistochemistry (IHC)

Product Details

Sequence:	YPYDVPDYA
Clone:	HA-C5
Isotype:	IgG3
Purification:	Purified

Target Details

Target:	HA-Tag
Alternative Name:	HA Tag (HA-Tag Products)
Target Type:	Tag

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Restrictions:	For Research Use only

Handling

Storage:	4 °C/-20 °C/-80 °C
Storage Comment:	Lyophilized antibodies can be kept at 4ºC for up to 3 months and should be kept at -20ºC for long-term storage (2 years). To avoid freeze-thaw cycles, reconstituted antibodies should be aliquoted before freezing for long-term (1 year) storage (-80ºC) or kept at 4ºC for short-term usage (2 months).

Publications

Product cited in:	Grzeschik, Hinz, Könning, Pirzer, Becker, Zielonka, Kolmar: "A simplified procedure for antibody engineering by yeast surface display: Coupling display levels and target binding by ribosomal skipping." in: **Biotechnology journal**, Vol. 12, Issue 2, (2017) (PubMed).

Validation report #100058 for Western Blotting (WB)

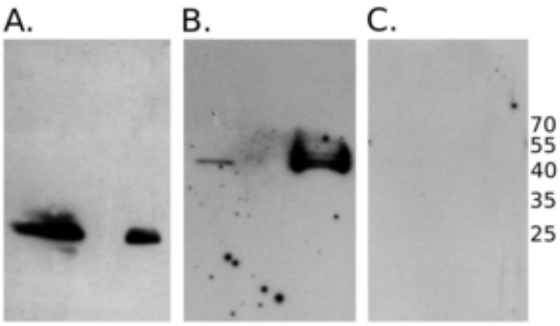
Successfully validated (Western Blotting (WB))

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

Report Number: 100058

Date: Sep 16 2016

Target:	HA-Tag
Lot Number:	16631508212-P
Method validated:	Western Blotting (WB)
Positive Control:	HA-fusion proteins expressed in NIH/3T3 cells
Notes:	Passed, regarding sensitivity and specificity. The HA-tag antibody ABIN2443910 does specifically recognize HA-tagged proteins in whole cell lysates from human tissue culture cells with no obvious background staining.
Primary Antibody:	ABIN2443910
Secondary Antibody:	Rabbit anti-Mouse IgG (whole molecule), HRP-linked (Sigma-Aldrich, A9044, lot number: 034M4761)
Protocol:	• Cultured NIH/3T3 cells heterologously expressing either a 26kDa (Fig. A) or 43kDa (Fig. B) HA-tag-fusion protein were rinsed with PBS and lysed in 6M Urea/20mM Tris. • 40µg total protein were used for Western blot analysis. • Proteins were denatured and separated on 10% SDS-PAGE (Laemmli 1970) and blotted to Amersham Protran Premium 0.2 NC (GE Healthcare, 10600004, lot A10043108) (Towbin et al., 1979). • Blocking of membrane in 5% skim milk in TBST (50 mM Tris-HCl, pH 7.4, 150mM NaCl, 0.1% Tween 20) for 30min at RT. • Incubation with primary antibody ABIN2443910 diluted 1:600 in 5% skim milk in TBST overnight at 4°C. • Washing in TBST for 30min at RT. • Incubation with secondary antibody secondary antibody: rabbit-anti-mouse IgG (whole molecule), HRP-linked (Sigma-Aldrich, A9044, lot 034M4761) diluted 1:10000 in 5% skim milk in TBST for 45min at RT. • Washing in TBST for 30-45min at RT. • Chemiluminescence detection using Clarity Western ECL Substrate (BioRad, 170-5061) according to the supplier's recommendations and image capture via X-ray films.
Experimental Notes:	A dilution factor <1000 is recommended for Western blot on cellular lysates with ABIN2443910.



Validation image no. 1 for anti-HA-Tag antibody (ABIN2443910)

HA-tagged fusion proteins were expressed in NIH/3T3 cells. Whole cell lysates were separated by SDS-PAGE and the tagged protein detected using anti HA-tag antibody ABIN2443910 diluted 1:600. Expected molecular mass of the fusion proteins were approximately 26kDa (A.) and 43kDa (B.) respectively. The left lane corresponds in both blots A. and B. to the pellet, the right to the supernatant after centrifugation. Exposition time for image capture on X-ray films: 1min (A.) and 30min (B.). For C., samples were prepared as for B. but the anti HA-tag antibody ABIN2443910 was diluted 1:1000. Exposure time: 15min.

Validation report #100059 for Immunocytochemistry (ICC)

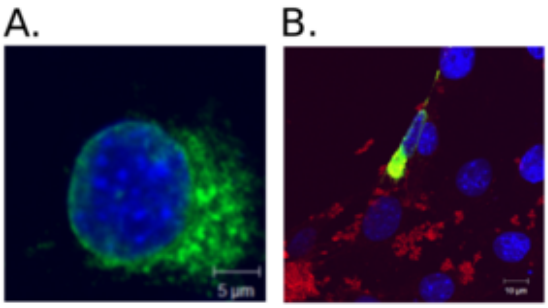
Successfully validated (Immunocytochemistry (ICC))

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

Report Number: 100059

Date: Sep 16 2016

Target:	HA-Tag
Lot Number:	16631508212-P
Method validated:	Immunocytochemistry (ICC)
Positive Control:	HA-fusion proteins transiently expressed in NIH/3T3 cells
Notes:	Passed, regarding sensitivity and specificity. The antibody specifically detects the HA-fusion protein in immuno-cytochemical experiments. No obvious background staining was found.
Primary Antibody:	ABIN2443910
Secondary Antibody:	MFP488 goat anti-mouse IgG (H+L) (MoBiTec, MFP-A1029, lot 2803101)
Protocol:	• NIH/3T3 cells (ATCC) were grown on cover slips in DMEM, 10% fetal bovine serum, 5% penicillin/streptomycin (all Gibco) at 37°C in 5% CO_2. • Cells were transfected using Metafectene Pro (Biontex, T040-1.0, lot AD1.13) according to the manual with a plasmid encoding an HA-tag fusion protein that is expected to localize to the nuclear membrane and the cytoplasm. • 24h post-transfection cells were fixed in 3.7% paraformaldehyde in PBS for 15min and processed for immuno-cytology. • Unspecific binding sites were blocked in PBST (phosphate buffered saline containing 0.15% bovine serum albumin, 0.1% Tween-20) for 1h. • Cells were incubated with the primary antibody ABIN2443910 diluted 1:100 in PBS at 4°C overnight. • Cells were washed in TBST (50mM Tris-HCl, pH 7.4, 150mM NaCl, 0.1% Tween 20) for 15min. • Incubation with the secondary antibody MFP488 goat anti-mouse IgG (H+L) (MoBiTec, MFP-A1029, lot 2803101) diluted 1:600 in PBS and DAPI (stock solution 10µg/µl, diluted 1:500) for 1h at 37°C. • Cells were washed in TBST for 15min. • Cells were embedded in Fluorescent Mounting Medium (Dako, S3023, lot 10090890). • Images were taken by confocal microscopy (LSM 510, Zeiss), and processed using Adobe Photoshop 5.0.
Experimental Notes:	none



Validation image no. 1 for anti-HA-Tag antibody (ABIN2443910)

HA-tagged fusion proteins were expressed in NIH/3T3 cells. The protein of interest is expected to localize to the nuclear membrane and the cytoplasm. A. The fusion protein was revealed via its HA-tag at the nuclear membrane and in the cytoplasm using ABIN2443910 (green; DAPI counterstain in blue). B. Co-staining of the fusion protein with using using anti-HA tag antibody ABIN2443910 (green) and an anti-myc-tag antibody (red; unspecific background staining caused by the transfection reagent). DAPI staining was used to visualize the nucleus (blue).