

Datasheet for ABIN2798885
anti-FAU antibody (N-Term)

3 Validations

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Overview

Quantity:	400 µL
Target:	FAU
Binding Specificity:	AA 1-30, N-Term
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This FAU antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (IHC)

Product Details

Purpose:	Rabbit Anti-Human FAU (N-term) Antibody
Immunogen:	This FAU antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 1-30 amino acids from the N-terminal region of human FAU.
Isotype:	Ig Fraction

Target Details

Target:	FAU
Alternative Name:	FAU (FAU Products)
Background:	Target Description: FUBI is the cellular homolog of the fox sequence in the Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV). It is a fusion protein consisting of the ubiquitin-like protein fubi at the N terminus and ribosomal protein S30 at the C terminus. It has been proposed that

Target Details

the fusion protein is post-translationally processed to generate free fubi and free ribosomal protein S30. Fubi is a member of the ubiquitin family, and ribosomal protein S30 belongs to the S30E family of ribosomal proteins. Whereas the function of fubi is currently unknown, ribosomal protein S30 is a component of the 40S subunit of the cytoplasmic ribosome.

Gene Symbol: FAU

Molecular Weight: 7760 Da

Gene ID: 2197

UniProt: [P35544](#)

Application Details

Application Notes: Western Blot, Immunohistochemistry

Recommended Dilutions

WB: 1:1000, IHC: 1:50-100 Western blot analysis of FUBI Antibody (N-term) polyclonal antibody, which was peroxidase-conjugated to the secondary antibody, followed by DAB staining. This data demonstrates the use of this antibody for immunohistochemistry, clinical relevance has not been evaluated.

Restrictions: For Research Use only

Handling

Format: Liquid

Concentration: 2.0 mg/mL

Storage: 4 °C, -20 °C

Storage Comment: 2-8°C (short-term), -20°C (long-term)



Successfully validated (Western Blotting (WB))

by [Institute for Biochemistry, Charité – Universitätsmedizin Berlin](#)

Report Number: 103222

Date: Aug 13 2018

Target:	FAU
Lot Number:	RAY8070723
Method validated:	Western Blotting (WB)
Positive Control:	Mouse tissue panel
Notes:	Passed, but low expression levels of endogenous FAU in most cell tissues tested.
Primary Antibody:	ABIN2798885
Secondary Antibody:	IRDye88CW goat anti-rabbit IgG (H+L) antibody (LI-COR, 926-32211)
Protocol:	<ul style="list-style-type: none"> Remove tissues from C57BL/6 male mice of 6 to 12 months of age, anaesthetized using isoflurane and killed by dislocation of the upper cervical spinal column. Freeze removed tissues in liquid nitrogen. Homogenize brain, heart, liver, lung, kidney, spleen, skeletal muscle and fat tissue using a FastPrep instrument (Savant) in 4-6 volumes of ice-cold lysis buffer containing 20mM HEPES pH7.4, 1% Triton X-100 (v/v), 5% glycerol (v/v), 8mM EDTA, 2mM EGTA, 'Complete' protease inhibitor cocktail (Roche, Cat. No. 11873580001, Lot 31880700). Centrifuge tissue extracts at 16000rcf at for 10min at 4°C. Collect supernatant and determine protein concentration using Bradford reagent and bovine serum albumin as a standard. Denature proteins for 5min at 95°C in 1x Laemmli SDS sample buffer. Separate 20µg of total protein per lane on a denaturing 16% Tris-glycine mini gels (Bio-Rad, freshly cast 8.3cm x 7.3cm for Mini Protean Tetra Cell) using Tris-glycine running buffer (Tris: Fisher Scientific, BP152-5, lot 171324; Glycine: AppliChem, A1067, lot 7A015185) in a Mini Protean Tetra Cell for 75min at 160V. Transfer proteins onto nitrocellulose membrane membrane (LI-COR, 926-31092, lot 25452956) by semi-dry blotting (Bio-Rad Trans-Blot SD) using discontinuous Tris-CAPS buffer system. Block membrane either with Rotiblock (Carl Roth, A151.4, lot 297261307), 5% milk in PBS, or 1% BSA in PBS for 45min at RT. Incubation with primary rabbit anti-FAU antibody (antibodies-online, ABIN2798885, lot RAY8070723) diluted 1:1000 in PBS/0.1% Tween 20 ON at 4°C. Wash membrane 3x for 10min with PBS/0.1% Tween 20. Incubate membrane with secondary IRDye88CW goat anti-rabbit IgG (H+L) antibody (LI-COR,

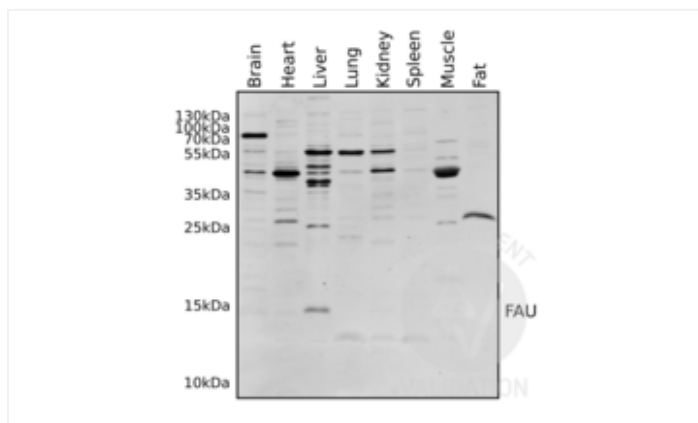
Validation report #103222 for Western Blotting (WB)

926-32211) diluted 1:10000 in PBS/0.1% Tween 20 for 60min at RT.

- Wash membrane 3x for 10min with PBS.
- Acquire image on a LI-COR Odyssey CLX scanner.

Experimental Notes: Detection of FAU (14kDa; full-length gene product: ubiquitin-like modifier FUBI + ribosomal protein S30) predominantly in liver extracts using Rotiblock as blocking reagent.

Image for Validation report #103222



Validation image no. 1 for anti-Finkel-Biskis-Reilly Murine Sarcoma Virus (FBR-MuSV) Ubiquitously Expressed (FAU) (AA 1-30), (N-Term) antibody (ABIN2798885)

Western blot analysis of various mouse tissue lysates using ABIN2798885.



Successfully validated (Western Blotting (WB))

by [Institute for Biochemistry, Charité – Universitätsmedizin Berlin](#)

Report Number: 103412

Date: Aug 13 2018

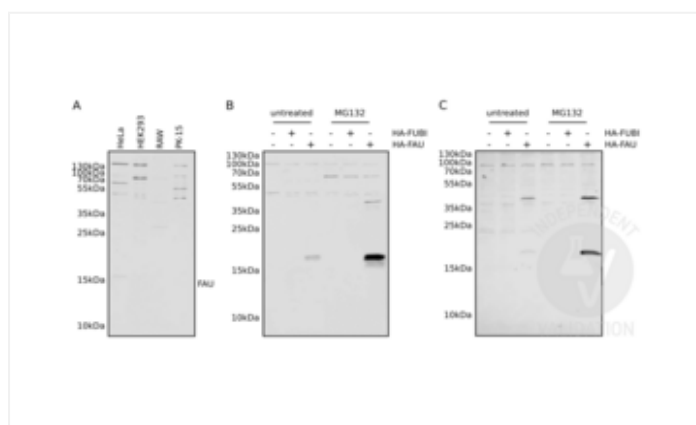
Target:	FAU
Lot Number:	RAY8070723
Method validated:	Western Blotting (WB)
Positive Control:	Endogenous FAU in extracts from human (HeLa, HEK293), murine (RAW), and porcine (PK-15) secondary cells HeLa cells transfected with HA-tagged FAU in vector pcDNA3.1
Negative Control:	HeLa cells transfected with empty plasmid vector pcDNA3.1
Notes:	Passed. ABIN2798885 specifically detects overexpressed FAU by western blotting.
Primary Antibody:	ABIN2798885
Secondary Antibody:	IRDye88CW goat anti-rabbit IgG (H+L) antibody (LI-COR, 926-32211)
Protocol:	<ul style="list-style-type: none"> Grow HeLa, HEK293, and RAW264.7 cells in DMEM (Thermo Fisher Scientific, 41965-039, lot 1924339) supplemented with 10% FBS (Merck Millipore, S0115, lot 1248D) and antibiotics (1x Penicillin-Streptomycin, Thermo Fisher Scientific, 15140-122, lot 1970744), at 37°C and 7% CO₂ in 6-well plates. Grow PK-15 cells in DMEM supplemented 5% FBS and antibiotics. Transfect 5x10⁵ HeLa cells/well with plasmids expressing either HA-FUBI, HA-FAU (FUBI+S30), or empty pcDNA3.1 vector using X-Treme Gene HP transfection reagent (Roche, 06366236001, lot 28049700; 3µg plasmid/well, ratio DNA to X-Treme Gene HP 1:1) following the manufacturer's instructions. Optional: treat cells with 2.5µM proteasome inhibitor MG132 (Biozol, CAY-10012628) for 16h before lysis. Lyse cells in ice-cold lysis buffer containing 20mM HEPES pH 7.4, 1% Triton X-100 (v/v), 5% glycerol (v/v), 8mM EDTA, 2mM EGTA, Complete protease inhibitor cocktail (Roche, 11873580001, lot 31880700). Centrifuge lysates at 16000rcf for 10min at 4°C to pellet cell debris and collect supernatant. Determine protein concentration using Bradford reagent (Thermo Fisher Scientific, 1856210, lot MH160588) and BSA as a standard. Denature proteins for 5min at 95°C in 1x Laemmli SDS sample buffer. Separate 20µg of total protein per lane on a 16% Tris-glycine mini gels (Bio-Rad, freshly cast 8.3cm x 7.3cm for Mini Protean Tetra Cell) using Tris-glycine running buffer (Tris: Fisher Scientific, BP152-5, lot 171324; Glycine: AppliChem, A1067, lot 7A015185) in a Mini Protean Tetra Cell for 75min at 160V.

- Transfer proteins onto nitrocellulose membrane (LI-COR, 926-31092, lot 25452956) by semi-dry blotting (Bio-Rad Trans-Blot SD) using discontinuous Tris-CAPS buffer system (Bio-Rad; CAPS: Sigma-Aldrich, C2632, lot SLBR8754).
- Block membrane either with Rotiblock (Carl Roth, A151.4, lot 297261307), 5% milk in PBS, or 1% BSA in PBS for 45min at RT.
- Incubation with primary
 - rabbit anti-FAU antibody (antibodies-online, ABIN2798885, lot RAY8070723) diluted 1:1000 in PBS/0.1% Tween 20 ON at 4°C or
 - rabbit anti-HA antibody (Abcam, ab91110, lot GR235874-6) diluted 1:5000 in PBS/0.1% Tween 20 ON at 4°C.
- Wash membrane 3x for 10min with PBS/0.1% Tween 20.
- Incubate membrane with secondary IRDye88CW goat anti-rabbit IgG (H+L) antibody (LI-COR, 926-32211) diluted 1:10000 in PBS/0.1% Tween 20 for 1h at RT.
- Wash membrane 3x for 10min with PBS.
- Acquire image on a LI-COR Odyssey CLX scanner.

Experimental Notes:

- No prominent immuno-signal for detected by Western blotting on extracts of tested secondary cell lines of human (HeLa, HEK293), murine (RAW), and porcine (PK-15) origin.
- HA-tagged FAU ectopically overexpressed in HeLa cells is readily detected using anti-FAU ABIN2798885.
- The presumed N-terminal cleavage product (ubiquitin-like modifier FUBI) of the FAU gene (FUBI + ribosomal protein S30) is not expressed in sufficient amounts to be detected by Western blotting. Even using a plasmid vector encoding HA-tagged ubiquitin-like modifier FUBI does not results in a proper expression of this protein.

Image for Validation report #103412



Validation image no. 1 for anti-Finkel-Biskis-Reilly Murine Sarcoma Virus (FBR-MuSV) Ubiquitously Expressed (FAU) (AA 1-30), (N-Term) antibody (ABIN2798885)

A. Western blot analysis on cell extracts from cultured human (HeLa, HEK293), murine (RAW), and porcine (PK-15) cells using ABIN2798885. B. Western blot on extracts from HeLa cells with (+) or without (-) ectopical expression of HA-tagged FAU or FUBI. Cells were treated with proteasome inhibitor (MG132) or not (untreated). C. Western blot of the same extracts as B. using an HA-tag antibody. Blocking in all images with 5% milk in PBS.



Successfully validated (Immunoprecipitation (IP))

by [Institute for Biochemistry, Charité – Universitätsmedizin Berlin](#)

Report Number: 103413

Date: Aug 13 2018

Target:	FAU
Lot Number:	RAY8070723
Method validated:	Immunoprecipitation (IP)
Positive Control:	HeLa cells transfected with HA-tagged FAU in vector pcDNA3.1
Negative Control:	HeLa cells transfected with empty plasmid vector pcDNA3.1
Notes:	Passed. ABIN2798885 enriches overexpressed FAU by immunoprecipitation.
Primary Antibody:	ABIN2798885
Secondary Antibody:	IRDye88CW goat anti-rabbit IgG (H+L) antibody (LI-COR, 926-32211)
Protocol:	<ul style="list-style-type: none"> • Grow HeLa cells in DMEM (Thermo Fisher Scientific, 41965-039, lot 1924339) supplemented with 10% FBS (Merck Millipore, S0115, lot 1248D) and antibiotics (1x Penicillin-Streptomycin, Thermo Fisher Scientific, 15140-122, lot 1970744), at 37°C and 7% CO₂ 10cm dishes. • Transfect 3x10⁶ cells/dish with plasmids expressing either HA-FUBI, HA-FAU (FUBI+S30), or empty pcDNA3.1 vector using X-Treme Gene HP transfection reagent (Roche, 06366236001, lot 28049700; 18µg plasmid/well, ratio DNA to X-Treme Gene HP 1:1) following the manufacturer's instructions. • Lyse cells in ice-cold lysis buffer containing 20mM HEPES pH 7.4, 1% Triton X-100 (v/v), 5% glycerol (v/v), 8mM EDTA, 2mM EGTA, Complete protease inhibitor cocktail (Roche, 11873580001, lot 31880700). • Centrifuge lysates at 16000rcf for 10min at 4°C to pellet cell debris and collect supernatant. • Determine protein concentration using Bradford reagent (Thermo Fisher Scientific, 1856210, lot MH160588) and BSA as a standard. • Prepare aliquots of lysates for SDS-PAGE in 1x Laemmli sample buffer. • Immobilize 10µl rabbit anti-FAU antibody (antibodies-online, ABIN2798885, RAY8070723) at 2µg/µl on 10µl SureBeads Protein A Magnetic Beads (Bio-Rad, 161-4013, lot 0304JA-01) in 1ml PBS containing 0.1% Tween 20 for 30min at 4°C. • Incubate 1mg of protein lysate in a total volume of 1 ml with 10 µl of magnetic protein A SureBeads (Bio-Rad) coupled non-covalently with anti-FAU antibody ABIN2798885 at for 1h at 4°C. Use non-coupled beads as control. Optional: use pre-immune rabbit serum as control. • Wash beads 2x with 1ml of lysis buffer and 1x with PBS. • Add 50µl non-reducing Laemmli buffer and incubate beads for 10min at RT to elute bound

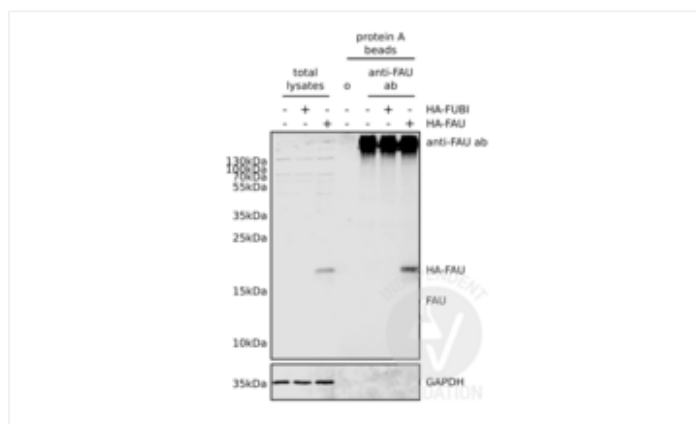
proteins.

- Separate 20µg of total protein and 1/5 of the IP sample per lane on a 16% Tris-glycine mini gels (Bio-Rad, freshly cast 8.3cm x 7.3cm for Mini Protean Tetra Cell) using Tris-glycine running buffer (Tris: Fisher Scientific, BP152-5, lot 171324; Glycine: AppliChem, A1067, lot 7A015185) in a Mini Protean Tetra Cell for 75min at 160 V.
- Transfer proteins onto nitrocellulose membrane (LI-COR, 926-31092, lot 25452956) by semi-dry blotting (Bio-Rad Trans-Blot SD) using discontinuous Tris-CAPS buffer system (Bio-Rad; CAPS: Sigma-Aldrich, C2632, lot SLBR8754).
- Block membrane with 5% milk in PBS for 45min at RT.
- Incubation with primary
 - rabbit anti-FAU antibody ABIN2798885 diluted 1:1000 in PBS/0.1% Tween 20 ON at 4°C or
 - rabbit anti-GAPDH antibody (Santa Cruz, sc-25778).
- Wash membrane 3x for 10min with PBS/0.1% Tween 20.
- Incubate membrane with secondary IRDye88CW goat anti-rabbit IgG (H+L) antibody (LI-COR, 926-32211) diluted 1:10000 in PBS/0.1% Tween 20 for 1h at RT.
- Wash membrane 3x for 10 min with PBS.
- Acquire image on a LI-COR Odyssey CLX scanner.

Experimental Notes:

- ABIN2798885 readily immunoprecipitates FAU protein, although overexpression might be required. Expression levels of endogenous FAU in HeLa cells appear to be too low to achieve proper enrichment by immunoprecipitation.
- The presumed N-terminal cleavage product (ubiquitin-like modifier FUBI) of the FAU gene (FUBI + ribosomal protein S30) is not expressed in sufficient amounts to be detected by Western blotting. Even using a plasmid vector encoding HA-tagged ubiquitin-like modifier FUBI does not results in a proper expression of this protein.

Image for Validation report #103413



Validation image no. 1 for anti-Finkel-Biskis-Reilly Murine Sarcoma Virus (FBR-MuSV) Ubiquitously Expressed (FAU) (AA 1-30), (N-Term) antibody (ABIN2798885)

Western blot analysis of HeLa cells expressing HA-tagged FAU (HA-FAU) or FUBI (HA-FUBI) using ABIN2798885. Extracts were submitted to immunoprecipitation using ABIN2798885 immobilized on protein A beads (protein A beads, anti-FAU ab). Total lysates or beads without immobilized antibody (protein A beads, o) were used as controls. GAPDH served as loading control.