Datasheet for ABIN948457

anti-SRSF5 antibody (AA 1-272)

1 Validation

2 Images

Go to Product page

Overview

Quantity:	50 µg
Target:	SRSF5
Binding Specificity:	AA 1-272
Reactivity:	Human
Host:	Mouse
Clonality:	Polyclonal
Conjugate:	This SRSF5 antibody is un-conjugated
Application:	Western Blotting (WB), Immunofluorescence (IF)

Product Details

Purpose:	Mouse polyclonal antibody raised against a full-length human SFRS5 protein.
Immunogen:	SFRS5 (NP_001034554.1, 1 a.a. ~ 272 a.a) full-length human protein.
Sequence:	MSGCRVFIGR LNPAAREKDV ERFFKGYGRI RDIDLKRGFG FVEFEDPRDA DDAVYELDGK ELCSERVTIE HARARSRGGR GRGRYSDRFS SRRPRNDRRN APPVRTENRL IVENLSSRVS WQDLKDFMRQ AGEVTFADAH RPKLNEGVVE FASYGDLKNA IEKLSGKEIN GRKIKLIEGS KRHSRSRSRS RSRTRSSSRS RSRSRSRSRK SYSRSRSRSR SRSRSKSRSV SRSPVPEKSQ KRGSSSRSKS PASVDRQRSR SRSRSRSVDS GN
Cross-Reactivity:	Human
Characteristics:	Antibody reactive against mammalian transfected lysate.

Target Details

Target:	SRSF5
Alternative Name:	SFRS5 (SRSF5 Products)
Background:	Full Gene Name: splicing factor, arginine/serine-rich 5 Synonyms: HRS,SRP40
Gene ID:	6430
NCBI Accession:	NM_001039465
Pathways:	Ribonucleoprotein Complex Subunit Organization

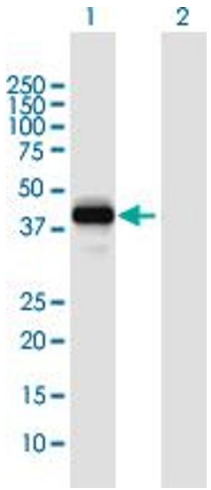
Application Details

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

Handling

Buffer:	In 1x PBS, pH 7.4
Handling Advice:	Aliquot to avoid repeated freezing and thawing.
Storage:	-20 °C
Storage Comment:	Store at -20°C or lower. Aliquot to avoid repeated freezing and thawing.

Images

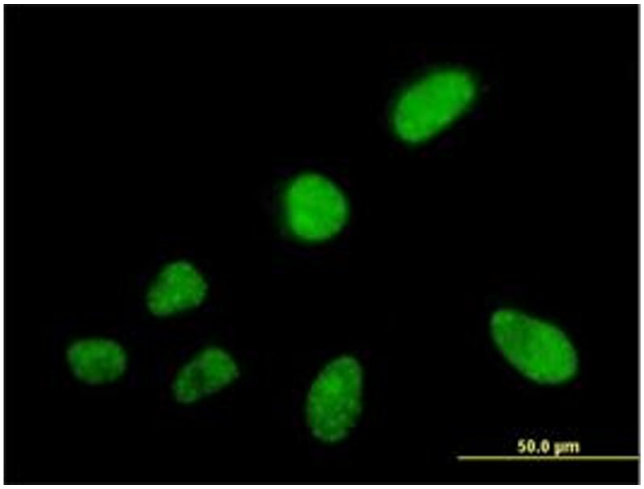


Western Blotting

Image 1. Western Blot analysis of SFRS5 expression in transfected 293T cell line by SFRS5 MaxPab polyclonal antibody.

Lane 1: SFRS5 transfected lysate(29.92 KDa).

Lane 2: Non-transfected lysate.



Immunofluorescence

Image 2. Immunofluorescence of purified MaxPab antibody to SFRS5 on HeLa cell. (antibody concentration 10 µg/mL)

Validation report #102043 for Western Blotting (WB)

Successfully validated (Western Blotting (WB))

by Laboratoire de Biologie et Modélisation de la Cellule, ENS Lyon

Report Number: 102043

Date: Nov 27 2017

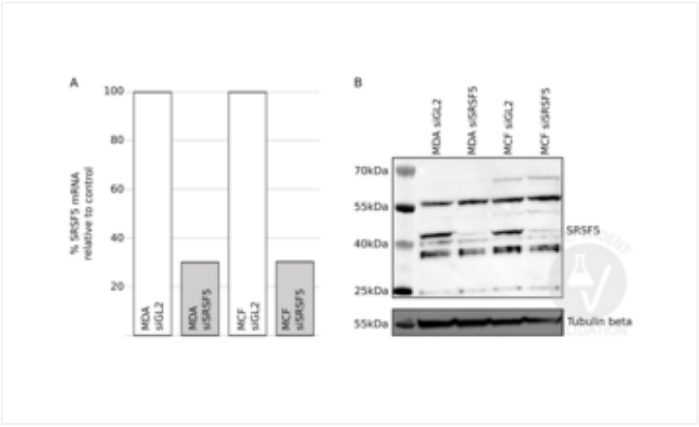
Target:	SRSF5
Lot Number:	11159
Method validated:	Western Blotting (WB)
Positive Control:	MDA-MB-231 control siRNA, MCF-7 control siRNA
Negative Control:	qPCR verified SRSF5 siRNA knockdown in MDA-MB-231 and MCF-7 cells
Notes:	ABIN948457 specifically reveals SRSF5 in human MDA-MB-231 and MCF-7 cell extracts but also several extraneous protein bands.
Primary Antibody:	ABIN948457
Secondary Antibody:	HRP-conjugated donkey anti-mouse antibody (Bethyl Laboratories, A90-137P)
Protocol:	• Grow cells in DMEM (MCF7: Gibco, 10938-025, lot 185560; MDA-MB-231: Gibco, 11880-028, lot 1894718) supplemented with FBS (Gibco, 131608-01, lot 42F6573K) and penicillin-streptomycin (Gibco, 15140-122, lot 1902417), and L-glutamine (Gibco, 25030-024, lot 1895805) 37°C and 5% CO_2. • Transfect 2.5x10^6 cells with amount 50nmol of siRNA (eurofins genomics) using Lipofectamine RNAiMAX reagent (Invitrogen, 13778030, lot 1863033) following the manufacturer´s instructions. • Grow cell for 48h. • Lyse 5x106 cells in 100µl of cold lysis buffer (50mM Tris-HCl, 400mM NaCl, 5mM EDTA pH8.0, 1% NP40, 0.2% SDS) containing 1x protease inhibitor cocktail (Roche, 05056489001). • Determine total protein content of the lysates using the Pierce BCA protein assay (ThermoFisher Scientific, 23225, lot sc246917). • Denature 30µg of total protein for 5min at 95°C in 30µl using NuPAGE Sample Reducing reagent 10x (Invitrogen, np0009, lot 1879834) and the LDS sample buffer (4X) (ThermoFisher Scientific, np0007, lot 1879570) and subsequently separate them on a denaturing gel Bis-Tris 4-12% Protein Gels (ThermoFisher Scientific, np0336box, lot 17061471) in MOPS migration buffer (ThermoFisher Scientific, NP0001, lot 1881690) for 1h at 160V. • Transfer proteins onto a Trans-Blot Turbo Mini Nitrocellulose membrane (Bio-Rad, 170-4158, lot 64126951) with a Transblot Turbo Transfer System (Bio-Rad, 1704150) for 7min at 1.3A and 25V. • Block the membrane with TBST (50mM Tris-HCl pH7.4-7.6, 150mM NaCl, 0.1% Tween 20)

containing 5% non-fat milk for 1h with slight agitation at RT.
- Incubation with primary mouse anti-human SRSF5 antibody (antibodies-online, ABIN948457, lot 11159) diluted to 1µg/µl in 5ml final volume in TBST + milk ON at 4°C.
- Wash membrane 2x for 10min with TBST.
- Incubation with secondary HRP-conjugated donkey anti-mouse antibody (Bethyl Laboratories, A90-137P) diluted 1:10000 in TBST + milk for 1h at RT.
- Wash membrane 2x for 10min with TBST.
- Reveal protein bands using ECL Prime Western Blotting Detection Reagent (GE healthcare RPN2236, lot 9794767) on a ChemiDoc MP Imaging System (Bio-Rad).

Experimental Notes:

- SRSF5 was depleted to almost 70% in both tested cell lines (Figure A). Quantities are normalized relative to the control siRNA (siGL2) and in comparison with a housekeeping gene (ribosomal 18S RNA, not shown). SRSF5 primers were previously validated on both cell lines. Efficacy of the primers are for the calculation estimated at 2.
- ABIN948457 specifically reveals a protein band with an apparent MW of approximately 45kDa in cell lysates which were transfected with control siRNA (Figure B). Post-translational modifications such as phosphorylation can influence the migration of SRSF5, making it appear bigger than it really is. The band disappears upon knockdown of the SRSF5 expression in cells transfected with an siSRSF5 construct. The antibody does also detect several other proteins at approximately 35-38kDa and 55kDa.

Image for Validation report #102043



Validation image no. 1 for anti-serine/arginine-Rich Splicing Factor 5 (SRSF5) (AA 1-272) antibody (ABIN948457)

A RT-qPCR verified depletion of SRSF5 transcription in MDA-MB-231 (MDA) and MCF-7 cells (MCF) subsequently to transfection with SRSF5 siRNA (siSRSF5) or control siRNA (siGL2). B Western blot analysis using ABIN948457 on cellular extracts with or without siRNA SRSF5 knock-down.