

Certificate of Analysis

Product: Affinity Purified Anti-Insulin Receptor Substrate 1 (IRS) pS307 [Rabbit]

Code: 600-401-445

Lot #: 17444

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 0.50 mg/ml (by UV absorbance at 280 nm)

Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Stabilizer: None

Preservative: 0.01% (w/v) Sodium Azide

Storage Conditions: Store vial at -20° C prior to opening. Dilute only prior to immediate use. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.

General Background: Insulin Receptor Substrate 1 (IRS1) acts as a signaling molecule for IL-4, insulin and insulin-like growth factor-I (IGF-I) receptors. When phosphorylated by the insulin receptor, IRS1 binds specifically to various cellular proteins containing SH2 domains such as phosphatidylinositol 3-kinase p85 subunit or GRB2. When bound, IRS1 typically activates phosphatidylinositol 3-kinase p85 subunit. IRS1 interacts with both the NPXY motif of tyrosine-phosphorylated IGF1R and the INSR through the PTB domain. Serine phosphorylation of IRS1 is a mechanism for insulin resistance. Ser-312 phosphorylation inhibits insulin action through disruption of IRS1 interaction with the

insulin receptor. Ser-307 phosphorylation is involved in the mechanism of insulin resistance in type 2 diabetes. Polymorphisms in IRS1 may be involved in the etiology of non-insulin-dependent diabetes mellitus (NIDDM).

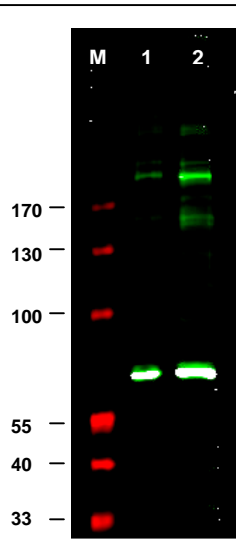


Figure 1. Western blot using Rockland's Affinity Purified anti-IRS1 pS307 antibody shows detection of a band at ~180 kDa believed to represent phosphorylated IRS1 (arrowhead). Lane 1 shows staining of human 293 cell lysate. Lane 2 shows staining of 293 cell lysate prepared from cells serum-starved for 18 h followed by treatment with 5 µg/ml of anisomycin for 30 min. The pronounced staining of the band at 180 kDa is not seen when the antibody was pre-incubated with immunizing peptide prior to reaction (data not shown). The identity of the intensely reactive bands at ~70 kD in both lane 1 and 2 is unknown, although these bands were also competed out by pre-incubation with the immunizing peptide. Approximately 25 µg of each lysate was separated on a 4-20% Tris-Glycine gel by SDS-PAGE and transferred onto nitrocellulose. After blocking with 5% goat serum, 0.5% BLOTTO in PBS, the membrane was probed with the primary antibody diluted to 1:250. Reaction occurred overnight at 4° C followed by washes and reaction with a 1:10,000 dilution of IRDye™ 800 conjugated Gt-a-Rabbit IgG [H&L] MX (611-132-122) for 45 min at room temperature (800 nm channel, green). Molecular weight estimation was made by comparison to prestained MW markers in lane M (700 nm channel, red). IRDye™ 800 fluorescence image was captured using the Odyssey® Infrared Imaging System developed by LI-COR. IRDye is a trademark of LI-COR, Inc. Other detection systems will yield similar results.

Application Note(s): This affinity purified antibody has been tested for use in ELISA and western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 180 kDa in size corresponding to phosphorylated IRS1 protein by western blotting in the appropriate cell lysate or extract. Minimal reactivity is observed against the non-phosphorylated form of the immunizing peptide. This antibody is phospho specific for pS307 of IRS1 protein.

Recommended Dilutions:	ELISA	1:2,000 - 1:10,000
	WESTERN BLOT	1:250 - 1:1,500
	IMMUNOHISTOCHEMISTRY	User Optimized
	OTHER APPLICATIONS	User Optimized

Purity and Specificity: This affinity-purified antibody is directed against the phosphorylated form of human IRS1 protein at the pS307 residue. The product was affinity purified from monospecific antiserum by immunoaffinity purification. Antiserum was first purified against the phosphorylated form of the immunizing peptide. The resultant affinity purified antibody was then cross-adsorbed

against the non-phosphorylated form of the immunizing peptide. Reactivity occurs against human IRS1 pS307 protein and the antibody is specific for the phosphorylated form of the protein. Reactivity with non-phosphorylated human IRS1 is minimal by ELISA. A BLAST analysis was used to suggest cross reactivity with IRS1 from human, mouse, rat, dog and vervet monkey based on 100% homology with the immunizing sequence. Partial reactivity is also expected against IRS1 from pig (94%), bovine (94%) and chicken (88%) sources. Reactivity of this antibody with IRS1 from other species is unknown.

Relevant Link(s): Swiss Prot: [P35568](#) NCBI Link [NP_005535](#)

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 298-316 of human IRS1 protein.

Protein Sequence: Human IRS1, 1242 aa, predicted MW 131.6 kDa

1	masppesdgf	sdvrkvgylr	kpksmhkrff	viraaseagg	parleyyene	kkwrhkssap
61	krsiplescf	ninkradskn	khlvlytrd	ehfaaadse	aeqdswyqal	lqlhnrakgh
121	hdgaaalgag	ggggscsgss	glgeagedls	ygdvppgpaf	kevwvilkp	kglgqtknli
181	gyrlcltsk	tisfvklmse	aaavvlqlmn	irrcghsenf	ffievgrsav	tgpgefwmqy
241	ddsvvaqnmh	etileamram	sdefprsk	qsssnscnpi	svplrhhln	npppsqvgl
301	rrsrtesita	tspasmvggk	pgsfrvrass	dgegtmsrpa	svdgspsvps	tnrthahrhr
361	gsarlhpln	hsrsimpas	rcpsatspv	slssstsg	gstdclfpr	rssasvsgsp
421	gdgflfssde	ygsspcdfsr	sfrsvtpdsl	ghtppargee	elsnyicmgg	kgpstltapn
481	ghylirsggn	ghrctpgtgl	gtspalagde	aasaadldnr	frkrthsagt	sptithqktp
541	sqssvasiee	ytemmpaypp	ggsggrlpg	hrhsafvptr	sypeeglemh	plerrgghhr
601	pdsstlhtdd	gymmpspgva	pvpgrksgsg	dymmpspksv	sapqqiinpi	rrhpqrvidp
661	gymmmspsgg	cspdigggps	sssssnnavp	sgtsgyglwt	ngvgghhshv	lphkpppves
721	sggkllpctg	dymnmspvgd	sntsspsdcy	ygpedpqhkp	visyyslprs	fkhtqrpgpe
781	eegarhqhrl	lstssgrlly	aatadssss	tssdslgggy	cgarleplsp	hphhqvlqph
841	lprkvdtaaq	tnsrlarptr	lslgdpkast	lprareqqqq	qqpllhpppe	kspgeyvnle
901	fgsdqsgyls	gpvafhssps	vrpcsqlqpa	preeetgte	ymkmdlgpgr	raawqestgv
961	emgrlpgapp	gaasicrptr	avpsrsgdym	tmqmscprqs	yvdtspaapv	syadmrtgia
1021	aeevslprat	maaassssaa	sasptgpqga	aelaahssll	ggpqqgggms	aftrvnlsn
1081	rnqsakvira	dpqgrrrhs	setfsstpsa	trvgntvpfg	agaavggggg	sssssedvkr
1141	hssasfenvw	lrpgeggap	kepalcgaa	gglenlnyi	dldlvkdfkq	cpqectpepq
1201	pppppphpq	lgsessstr	rssedsaya	sisfqkped	rq	

Reference(s):

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- Aguirre, V., Uchida, T., Yenush, L., Davis, R. and White, M.F. (2000) The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *J Biol Chem* **275** (12), 9047-9054.
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Related Products:

#W09-000-365	293 Whole Cell Lysate in SDS-PAGE Sample Buffer
#611-703-127	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
#611-132-122	IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
#KIA-003	MaxTag TM Anti-RABBIT IgG Kit for Immunoblotting
#MB-070	Blocking Buffer for Fluorescent Western Blotting
#B501-0500	BLOTTO
#BSA-30	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer)

USDA Certification: All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation.

Note: This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.

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