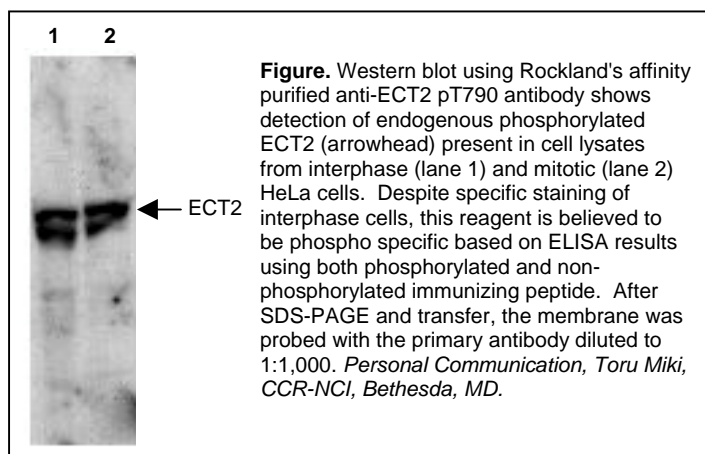


Certificate of Analysis**Product:** Affinity Purified Anti-ECT2 pT790 (Rabbit)**Code:** 600-401-935**Lot #:** 17548**Size:** 100 µg**Physical State:** Liquid (sterile filtered)**Antibody Concentration:** 1.31 mg/ml (by UV absorbance at 280 nm)**Buffer:** 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, 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.

Background Information: ECT2, also known as epithelial cell transforming sequence 2 oncogene, was originally isolated as a transforming gene from epithelial cells. ECT2 catalyzes guanine nucleotide exchange on the small GTPases, RhoA, Rac1, and Cdc42. ECT2 may be phosphorylated during G₂ and M phases, and phosphorylation may be required for its exchange activity. Unlike other known guanine nucleotide exchange factors for Rho GTPases, ECT2 exhibits nuclear localization in interphase, spreads throughout the cytoplasm in prometaphase, and is condensed in the midbody during cytokinesis. Expression of dominant-negative ECT2 or microinjection of affinity-purified anti-ECT2 antibody into interphase cells strongly inhibits cytokinesis. These results suggest that ECT2 is an important link between the cell cycle machinery and Rho signaling pathways involved in the regulation of cell division. Phosphorylation at T790 or S375 significantly affects the catalytic activity of ECT2.



Recommended Dilutions:	ELISA	1:10,000 - 1:40,000
	WESTERN BLOT	1:1,000 - 1:4,000
	IF MICROSCOPY	User Optimized
	OTHER APPLICATION	User Optimized

Application Note(s): This affinity-purified antibody has been tested for use in ELISA, western blot and immunoprecipitation. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 101 kDa in size by western blotting in the appropriate cell lysate or extract. Less than 2% reactivity is observed against the non-phosphorylated form of the immunizing peptide. This antibody is believed to be phospho specific for ECT2 phosphorylated at the pT790 residue. Experiments to generate more conclusive evidence are currently under preparation.

Purity and Specificity: This affinity-purified antibody is directed against the phosphorylated form of human ECT2 protein at the pT790 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 ECT2 protein at the pT790 residue. By ELISA the antibody is specific for the phosphorylated form of the protein. Reactivity with non-phosphorylated human ECT2 is minimal by ELISA, but western blot shows specific staining of interphase cell lysates, which are predicted to contain non-phosphorylated ECT2. Additional experiments are underway to clarify antibody specificity. A BLAST analysis was used to suggest cross reactivity with ECT2 protein from human, mouse, rat, zebrafish, chimpanzee, chicken, and dog based on 100% homology with the immunizing sequence. Reactivity against homologues from other sources is not known.

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 785-795 of human ECT2 protein.

Relevant Links: NCBI [NP_060568](#) Swis-Prot [Q9H8V3](#)

Protein Sequence: Human ECT2, 883 aa, predicted MW 100.0 kDa

1	maensvlst	tgrtsladss	ifdskvteis	kenlligsts	yveeempqie	trvilvqeag
61	kqeelikalk	dikvgfvkme	sveefeglds	pefenvfvvt	dfqdsvfndl	ykadcrvigp
121	pvlncsqkg	eplpfscrpl	yctsmmnlvl	cftgfrkkee	lvrlvtlvhh	mggvirkdfn
181	skvthlvanc	tqgekfrvav	slgtpimkpe	wiykawerrn	eqdfyaavdd	frnefkvppf
241	qdcilsflgf	sdeektnmee	mtemqggkyl	plgdercthl	vveenivkdl	pfepskklyv
301	vkqewfwgsi	qmdaragetm	yliekantpe	lkksvsmisl	ntpnsnrkrr	rlketlaqls
361	retdvspfpp	rkrpsaehsl	sigslldisn	tpessinygd	tpksctkssk	sstpvpqskq
421	arwqvakely	qtesnyvnll	atiiqlfqvp	leeegqrggp	ilapeeikti	fgsipdldfv
481	htkikddled	livnwdesks	igdifikysk	dlvktypfv	nffemsketi	ikcekqkprf
541	hafkinqak	pecgrqslve	llirpvqrlp	svallndlk	khtadenpdk	stlekaigsl
601	kevmthined	krkteaqkqi	fdvvyevdgc	panllsshrs	lvqrvetisl	gehpcdrgeq
661	vtlffndcl	eiarkrhkvi	gtfrsphgqt	rppaslkhih	lmpsqikkv	ldirededch
721	nafallvrpp	teqanvlisf	qmtsdelpke	nwlkmlcrhv	antickadae	nliytadpes
781	fevntkdmtds	ts rasraik	ktskkvtraf	sfsktpkral	rrealmtshgs	vegrpspsnd
841	khvmsrlsst	sslagipsps	lvslpsffer	rshltsrstt	hli	

General References:

Takashi Tatsumoto, Xiaozhen Xie, Rayah Blumenthal, Isamu Okamoto, and Toru Miki (1999) Human ECT2 Is an Exchange Factor for Rho GTPases, Phosphorylated in G2/M Phases, and Involved in Cytokinesis. *J. Cell Bio*, **147** (5), 921–927.

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#611-132-122	IRDye800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10	
#B501-0500	500 g	BLOTTO
#BSA-30	500 ml	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer)
#B304	10 ml	NORMAL GOAT SERUM (NGS)
#MB-070	Blocking Buffer for Fluorescent Western Blotting	
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#MB-070	Blocking Buffer for Fluorescent Western Blotting	

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