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Certificate of Analysis

Product: Affinity Purified Anti-CDC27 pT244 [Rabbit]

Code: 600-401-885

Lot #: 20108

Size: 100 µg

Physical State: Liquid (sterile filtered)

Antibody Concentration: 1.0 mg/ml (by UV absorbance at 280 nm)

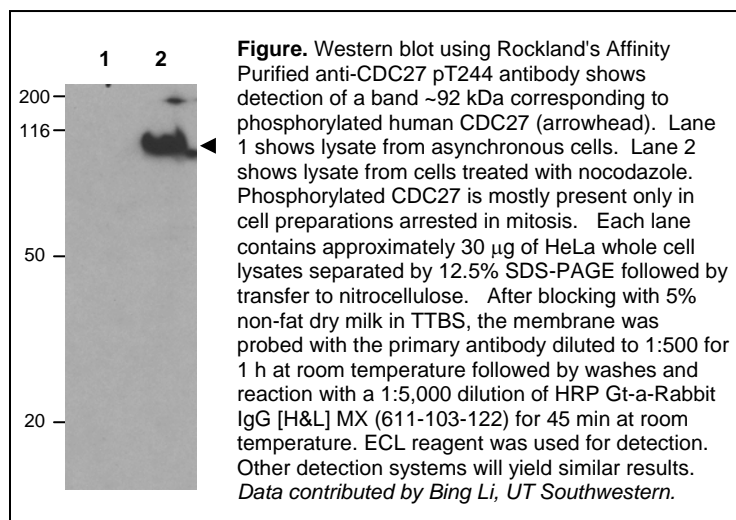
Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

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: Human CDC27 (also called Cell division cycle protein 27 homolog, CDC27Hs and H-NUC) shares strong similarity with *Saccharomyces cerevisiae* protein Cdc27. This protein is a component of anaphase-promoting complex (APC), which is composed of eight protein subunits and highly conserved in eukaryotic cells. APC catalyzes the formation of a cyclin B-ubiquitin conjugate that is responsible for the ubiquitin-mediated proteolysis of B-type cyclins. This protein and 3 other members of the APC complex contain the TPR (tetratricopeptide repeat), a protein domain important for protein-protein interaction. This protein was shown to interact with mitotic checkpoint proteins including Mad2, p55CDC and BUBR1, and thus may be involved in controlling the timing of mitosis.



Application Note(s): This affinity purified antibody has been tested for use in ELISA and by western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 92 kDa in size corresponding to CDC27 by western blotting in the appropriate cell lysate or extract. Less than 1% reactivity is observed against the non-phosphorylated form of the immunizing peptide. This antibody is phospho specific for pT244 of CDC27.

Recommended Dilutions:	ELISA	1:8,000 - 1:30,000
	WESTERN BLOT	1:300 - 1:2,000
	IF MICROSCOPY	User Optimized
	OTHER APPLICATIONS	User Optimized

Purity and Specificity: This affinity purified antibody is directed against the phosphorylated form of human CDC27 at the pT244 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 CDC27 pT244 protein and the antibody is specific for the phosphorylated form of the protein. Reactivity with non-phosphorylated human CDC27 is minimal by ELISA. The antibody does not cross-react with CDC27 phosphorylated at other sites. A BLAST analysis was used to suggest reactivity with this protein from human, rat, dog, bovine and chimpanzee based on 100% homology for the immunogen sequence. Cross reactivity with CDC27 protein from mouse, rat and chicken may occur as sequence homology varies by one amino acid residue in this sequence (90% homology). Cross reactivity with CDC27 homologues from other sources has not been determined.

Relevant Link(s): Swiss Prot: [P30260](#) NCBI Link [NP_001247](#)

Protein Sequence: Human CDC27, 824 aa, predicted MW 91.8 kDa

1	mtvlqepvqa	aivqalnhya	yrdavflaer	lyaevhseea	lfllatcyyr	sgkaykayrl
61	lkghscttpq	ckyllakccv	dlsklaeeg	ilsggvfnkq	kshddivtef	gdsactflsl
121	lghvycktdr	lakgsecyqk	slslnpflws	pfeslceige	kpdpdqtkf	tslqnfsncl
181	pnscttqvpn	hslshrqpvt	vitetpqdti	elnrlnless	nskyslntds	svsyidsavi
241	spdtvplgtg	tsilskvqvn	kpktgrslig	gpaalspltp	sfgilpletp	spgdgsylqn
301	ytntppvidv	pstgapskks	varigtgtgk	svfsqsgnsr	evtpilaqtq	ssgpqtsttp
361	qvlspitisp	pnalprssr	lftdssttk	enskkklmkf	ppkipnrktk	sktnkkggitq
421	pnindsleit	kldssiiseg	kistitpqiq	afnlqkaaaa	glmsllremg	kgylalcsyn
481	ckeainilsh	lpshhyntgw	vlcqjgrayf	elseymqaer	ifsevrrien	yrvevrmeys
541	ttlwhlqkdv	alsvlskdlit	dmdknspeaw	caagncflsq	rehdiaikff	qraiqvdpny
601	ayaytllghe	fvlteeldka	lacfrnairv	nprhynawyg	lgmiyykqek	fslaemhfqk
661	aldinpqssv	llchigvvqh	alkksekald	tlnkaividp	knplckfhra	svlfaneyk
721	salqeleeek	qivpkleslv	fliggvvykl	gqthlalmnf	swamldldpkg	annqikeaid
781	krylpddeep	itqeeqimgt	desqessmtd	addtqlhaae	sdef	

Immunogen: This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding aa 239-249 of Human CDC27.

General References:

Gabellini,D., Colaluca,I.N., Vodermaier,H.C., Biamonti,G., Giacca,M., Falaschi,A., Riva,S. and Peverali,F.A. (2003) Early mitotic degradation of the homeoprotein HOXC10 is potentially linked to cell cycle progression. *EMBO J.* **22** (14), 3715-3724.

Topper,L.M., Campbell,M.S., Tugendreich,S., Daum,J.R., Burke,D.J., Hieter,P. and Gorbisky,G.J. (2002) The dephosphorylated form of the anaphase-promoting complex protein Cdc27/Apc3 concentrates on kinetochores and chromosome arms in mitosis. *Cell Cycle* **1** (4), 282-292.

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Yu,H., Peters,J.M., King,R.W., Page,A.M., Hieter,P. and Kirschner,M.W. (1998) Identification of a cullin homology region in a subunit of the anaphase-promoting complex. *Science* **279** (5354), 1219-1222.

Ho,P.P. et al. (1995) Localization of the human homolog of the yeast cell division control 27 gene (CDC27) proximal to ITGB3 on human chromosome 17q21.3. *Somat. Cell Mol. Genet.* **21** (5), 351-355.

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Chen,P.L., Ueng,Y.C., Durfee,T., Chen,K.C., Yang-Feng,T. and Lee,W.H. (1995) Identification of a human homologue of yeast nuc2 which interacts with the retinoblastoma protein in a specific manner. *Cell Growth Differ.* **6** (2), 199-210.

Tugendreich,S., Boguski,M.S., Seldin,M.S. and Hieter,P. (1993) Linking yeast genetics to mammalian genomes: identification and mapping of the human homolog of CDC27 via the expressed sequence tag (EST) data base. *Proc. Natl. Acad. Sci. U.S.A.* **90** (21), 10031-10035.

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