

Certificate of Analysis

Product: IgG Fraction of Anti-Ubiquitin Activating Enzyme E1 [Rabbit]

Code: 200-401-888

Lot #: 16536

Size: 500 µg

Physical State: Lyophilized

Antibody Concentration: 5.0 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 4° C prior to restoration. Reconstitute with 0.1 ml of deionized water or equivalent. 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 restoration.

Background: Ubiquitin Activating Enzyme (E1), also known as A1S9 and UBE1, is responsible for the first step in ubiquitin-protein isopeptide bond formation. E1 catalyzes the activation of the C-terminal carboxyl group of ubiquitin by forming a high-energy thioester bond in an ATP-dependent manner. UBE1 is monomeric and contains two active sites within the E1 molecule, allowing it to bind two ubiquitin moieties at a time, with a new ubiquitin forming an adenylate intermediate as the previous one is transferred to the thiol site. Alternative splicing results in 2 transcript variants encoding the same protein, but with different 5' UTR. Isoform 1 has a different 5' noncoding exon compared to isoform 2. Both variants encode the same protein.

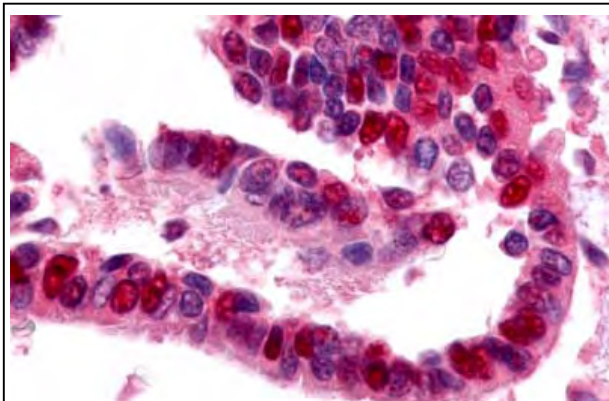
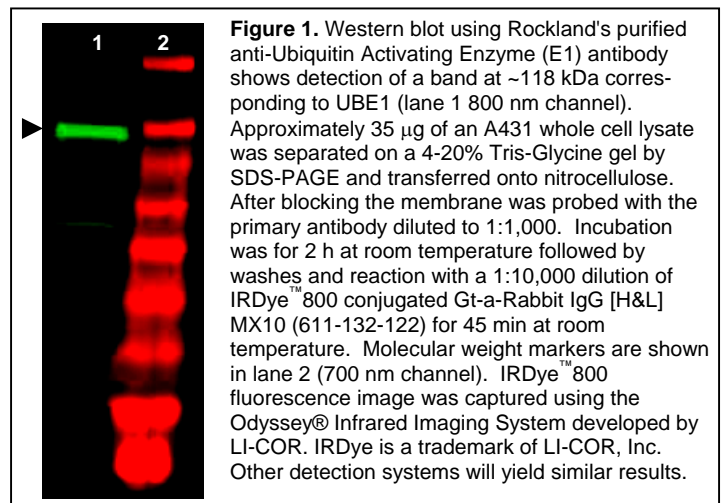


Figure 2. Immunohistochemistry. Rockland's Affinity Purified anti-Ubiquitin Activating Enzyme antibody was used at a 10 µg/ml to detect UBE1 in a variety of tissues including adrenal, breast, colon (epithelium), kidney, liver, lung (respiratory epithelium), ovary (oocyte and endothelium), pancreas (islet and exocrine), placenta, prostate (epithelium), skin (epithelium), spleen (lymphocytes), stomach (chief), testis, thymus, tonsil, and uterus (glandular, stroma). In many cells a punctate nuclear staining was observed. Other cells showed both cytoplasmic and nuclear staining. This image shows UBE1 staining of human lung tissue. Tissue was formalin-fixed and paraffin embedded. *Personal Communication, Tina Roush, LifeSpanBiosciences, Seattle, WA.*

e-mail: info@rockland-inc.com

Application Note(s): This purified antibody has been tested for use in ELISA, immunohistochemistry and western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band at ~118 kDa in size corresponding to UBE1 by western blotting in the appropriate cell lysate or extract.

Recommended Dilutions:

ELISA	1:2,000 - 1:10,000
WESTERN BLOT	1:1,000 - 1:5,000
IMMUNOHISTOCHEMISTRY	2 µg/ml - 20 µg/ml
OTHER APPLICATIONS	User Optimized

Purity and Specificity: This purified antibody is directed against human Ubiquitin Activating Enzyme E1 protein. The product was purified from monospecific antiserum by a multi step procedure. A BLAST analysis was used to suggest that this antibody would react with Ubiquitin Activating Enzyme E1 protein from human (100%) rabbit (96%), mouse (95%), rat (95%) and dog (93%) based on a high degree of sequence homology. Cross reactivity against this protein from other sources has not been determined.

Relevant Link(s):

Swis-Prot [P22314](#)

NCBI Link [NP_003325](#)

Visit our website at: www.rockland-inc.com

Protein Sequence: Human Ubiquitin Activating Enzyme E1, 1058 aa, predicted MW 117.8 kDa

1	msspslskr	rvsgdpkpg	sncspaqsvl	sevpvsptng	makngseadi	deglysrqly
61	vlgheamkrl	qtssvlvsgl	rglgsveiakn	iilggvkavt	lhdqgtaqwa	dlssqfylre
121	edignkraev	sqprlaelns	yvpvtaytgp	lvedflsgfq	vvvtntple	dqlrvgefch
181	nrgiklvvad	trglfgqlfc	dfgeemildt	snggeplsam	vsmvtkdnpq	vvtcldearh
241	gfesgdvsvf	sevqgmveln	gnqpmeikvl	gpytfsicdt	snfsdyirgg	ivsqvkvppk
301	isfkslvasl	aepdfvvtdf	akfsrpaqlh	igfqalhqfc	aqhgrpprpr	needaaelva
361	laqavnaral	pavqqnnlde	dlirklayva	agdlapinaf	igglaaqevm	kacsqgkmpj
421	mqwlyfdale	clpedkevit	edkclqrqr	ydgqvavfqs	dlqeklgkqk	yflvgagaig
481	cellknfami	glgcgeggei	ivtdmdtik	snlnrqflfr	pwdvtklksd	taaaavrqm
541	phirtshqn	rvgpdteriy	dddfqnlidg	vanaldnvda	rmymdrrcvy	yrkpllesgt
601	lgtkgnvqv	ipfltesyss	sqdppeksip	ictlknfna	iehtlward	efeglfkqpa
661	envnqyltdp	kfvrtlrla	gtqplevlea	vqrsvlvqrp	qtwadcvtwa	chhwhtqysn
721	nirqlhnp	pdqltssgap	fwsgpkrcph	pltfvnnpl	hldyvmaaan	lfaqtygltg
781	sqdraavaf	lqsvqvpeft	pksgvkihvs	dqelqsanas	vddsrlleek	atlpdpklp
841	gfkmypidfe	kdddsfnhmd	fivaasnra	enydipsadr	hkskliagki	ipaiaattaa
901	vvglvclely	kvvqghrql	sykngflnla	lpffgfsepl	aaphrhyynq	ewtlwdrfev
961	qglqpngeem	tlkqfldyfk	tehkleitml	sqgvsmlysf	fmpaaklker	ldqpmteivs
1021	rvskrkigrh	vralvlelcc	ndesgedvev	pyvrytir		

Immunogen: This purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a recombinant protein corresponding to full length Human Ubiquitin Activating Enzyme E1 fused with GST.

General References:

Beausoleil, S.A., Jedrychowski, M., Schwartz, D., Elias, J.E., Villen, J., Li, J., Cohn, M.A., Cantley, L.C. and Gygi, S.P. (2004) Large-scale characterization of HeLa cell nuclear phosphoproteins. *Proc. Natl. Acad. Sci. U.S.A.* **101** (33), 12130-12135.

Saito, Y., Doi, K., Yamagishi, N., Ishihara, K. and Hatayama, T. (2004) Screening of Hsp105alpha-binding proteins using yeast and bacterial two-hybrid systems. *Biochem. Biophys. Res. Commun.* **314** (2), 396-402.

Bres, V., et al. (2003) A non-proteolytic role for ubiquitin in Tat-mediated transactivation of the HIV-1 promoter. *Nat. Cell Biol.* **5** (8), 754-761.

Stephen, A.G., Trausch-Azar, J.S., Ciechanover, A. and Schwartz, A.L. (1996) The ubiquitin-activating enzyme E1 is phosphorylated and localized to the nucleus in a cell cycle-dependent manner. *J. Biol. Chem.* **271** (26), 15608-15614.

Nagai, Y., Kaneda, S., Nomura, K., Yasuda, H., Seno, T. and Yamao, F. (1995) Ubiquitin-activating enzyme, E1, is phosphorylated in mammalian cells by the protein kinase Cdc2. *J. Cell. Sci.* **108** (PT 6), 2145-2152.

Cook, J.C. and Chock, P.B. (1995) Phosphorylation of ubiquitin-activating enzyme in cultured cells. *Proc. Natl. Acad. Sci. U.S.A.* **92** (8), 3454-3457.

Related Products: (visit www.rockland-inc.com for a complete listing of Ubiquitin related antibodies)

#200-401-431	IgG fraction of Anti-UBIQUITIN (Rabbit)
#200-401-438	IgG fraction of Anti-Human ISG15 (Rabbit)
#200-401-441	IgG fraction of Anti-Human SUMO (Rabbit)
#W09-000-361	A431 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
#MB-070	Blocking Buffer for Fluorescent Western Blotting
#KIA-003	MaxTag TM Anti-RABBIT IgG Kit for Immunoblotting

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.