

Standard Operating Procedure

Preparation of active ROCK-II/ROK α

Enzyme description:-	Active ROCK-II/ROK α
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system (BEVS)/Insect cells
<u>Tag:-</u>	His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose.
<u>Expression level:-</u>	3-5 mg/L
<u>Molecular mass:-</u>	67 kDa by SDS-PAGE
<u>Purity:-</u>	>85%
<u>Contaminants:-</u>	The preparation contains several minor degradation products.
<u>Activation protocol:-</u>	N/A – constitutively active when purified from insect cells.
<u>Enzyme storage buffer:-</u>	50 mM Tris/HCl pH 7.5, 50% glycerol, 150 mM NaCl, 0.1 mM EGTA, 0.1 % β -mercaptoethanol, 0.02% Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.
<u>Storage temperature:-</u>	-20°C.

CLONE DATA SHEET – rat ROCK-II/ROK α

<u>Protein</u>	ROK alpha (2-543) Rat
<u>Accession number</u>	U38481
<u>Tags</u>	His(6) and HA () amino-terminal
<u>Baculovirus-expressed protein</u>	MSYYHHHHHHHDYDIPTTENLYFQGAMGSEFATMYPYDV PDYAPGAPEAAAGDGAGAGRQRKLEALIRDPRSPINVE SLDGLNSLVLDLDFPALRKNKNIDNFLNRYEKIVKKIR GLQMKAEDYDVVKVIGRGAFGEVQLVRHKASQKVYA MKLLSKFEMIKRSDSAFFWEERDIMAFAFANSPWVQVLC AFQDDRYLYMVMMEYMPGGDLVNLMSNYDVPEKWAK FYTAEVVLALDAIHSMGLIHRDVKPDNMLLDKHGHLK LADFGTCMKMDETGMVHCDTAVGTPDYISPEVLKSQ GGDGYYGRECDWWSVGVLFEMLVGDTPFYADSLVG TYSKIMDHKNSLCFPEDTEISKHAKNLICAFITDREVRL GRNGVEEIKQHPFFKNDQWNWDNIRETAAPVVPELSS DIDSSNFDDIEDDKGDVETFPKAFVGNQLPFIGFTYFR ENLLSDSPPCRENDAIQTRKSEESQEIQKKLYALEEHL SEVQAKEELEQKCKSINTRLEKTAKELEEEITFRKNVEST LRQLEREKALLQHKNAEYQRKADHEADKKRNLENDV NSLKDQLEDLKKRNQSSQI
<u>Native sequence</u>	P43 of the fusion-protein is P1 of ROK alpha (QHP should be SAS according to sequence of given accession number) – We believe that the original sequence in the database is incorrect, as the sequence from other species is similar to our sequence. There is a His(6) tag at residues 5- 10 and an HA-tag (YPYDVPDYA) at residues 34-42 of the fusion protein.
<u>Protease cleavage site</u>	ENLYFQ (rTEV protease) residues 18 – 23.

**ORF in
baculovirus**

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCA
ACGACCGAAAACCTGTATTTTCAGGGCGCCATGGGATCCGAATTC
GCCACCATGTACCCATACGATGTGCCAGATTACGCCCCGGCGCC
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GACAAGAAACGGAATTTGGAAAATGATGTTAACAGCTTAAAGAT
CAACTTGAAGATTTGAAGAAAAGAAACCAGAGCTCTCAGATATAA