Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active VPS34 [2 - 887]

Enzyme description:- VPS34 [2 - 887]

Clone number:- DU 3303

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6) and HA

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic 106, 453.88 daltons
Average Mass 106, 521.77 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.05

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine

Storage temperature:- -70 °C

Assay:- ADP Glo

Assay buffer:-
20 mM Tris-HCl, 67 mM KCl, 1 mM EDTA, 1 mM DTT, 0.05 mg/ml BSA, 10 mM $MgCl_2$

Substrate:-
PI Final concentration: 0.04 mM

Division of Signal Transduction Therapy

Clone Data Sheet

VPS34 [2 – 887]

Protein	VPS34 [2 - 887]
Clone number	DU 3303
Species	Human
Accession number	NM_002647
Tags	N-terminal His(6) and HA (YPYDVPDYA)
Baculovirus Expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMDPEFATMYPYDVPDYA**GEAEKFH** **YIYSCDLDINVQLKIGSLEGKREQKSYKAVLEDPMLKFSGLYQETCSDL** **YVTCQVFAEGKPLALPVRTSYKAFSTRWNWNEWLKLPVKYPDLPRNAQV** **ALTIWDVYGPGKAVPVGGTTVSLFGKYGMFRQGMHDLKVWPNVEADGSE** **PTKTPGRTSSTLSEDQMSRLAKLTKAHRQGHMVKVDWLDRLTFREIEMI** **NESEKRSSNFMYLMVEFRCVKCDDKEYGIVYYEKDGDESSPILTSFELV** **KVPDPQMSMENLVESKHHKLARSLRSGPSDHDLKPNAATRDQLNIIVSY** **PPTKQLTYEEQDLVWKFRYYLTNQEKALTKFLKCVNWDLPQEAKQALEL** **LGKWKPMDVEDSLELLSSHYTNPTVRRYAVARLRQADDEDLLMYLLQLV** **QALKYENFDDIKNGLEPTKKDSQSSVSENVSNSGINSAEIDSSQIITSP** **LPSVSSPPPASKTKEVPDGENLEQDLCTFLISRACKNSTLANYLYWYVI** **VECEDQDTQQRDPKTHEMYLNVMRRFSQALLKGDKSVRVMRSLLAAQQT** **FVDRLVHLMKAVQRESGNRKKKNERLQALLGDNEKMNLSDVELIPLPLE** **PQVKIRGIIPETATLFKSALMPAQLFFKTEDGGKYPVIFKHGDDLRQDQ** **LILQIISLMDKLLRKENLDLKLTPYKVLATSTKHGFMQFIQSVPVAEVL** **DTEGSIQNFFRKYAPSENGPNGISAEVMDTYVKSCAGYCVITYILGVGD** **RHLDNLLLTKTGKLFHIDFGYILGRDPKPLPPPMKLNKEMVEGMGGTQS** **EQYQEFRKQCYTAFLHLRRYSNLILNLFSLMVDANIPDIALEPDKTVKK** **VQDKFRLDLSDEEAVHYMQSLIDESVHALFAAVVEQIHKFAQYWRK**
Native sequence	Amino acids G2 – K887 (end) of human VPS34. Residue G43 of the fusion protein is equivalent to G2 of the native enzyme. The His(6) tag is located at residues 5 – 10 and the HA tag is located at residues 34 – 42..
Protease cleavage	rTEV (ENLYFQG) residues 18 – 24

Cloning sites *Eco*R1 of pFastBac HTa

Nucleotide of sequence of insert

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