Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-SAE1 / His-SAE2

Enzyme description:- SAE1 / SAE2 heterodimer (full length)

Clone number:- SC20972

Source:- Recombinant

Tag:- N-terminal GST on SAE1 and His_6 on SAE2

Purification method:- 1. Ni^{++}-Sepharose, 2.GSH-Sepharose

Expression level:- 0.4mg/L

Calculated molecular mass:-

Monoisotopic GST-SAE1 = 64700 Da, His-SAE2 = 73168 Da
Average Mass GST-SAE1 = 64740 Da, His-SAE2 = 73210 Da
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- GST-SAE1 = 5.66, His-SAE2 = 5.39

Purity:- 90%

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

Storage temperature:- -80ºC

Assay:-
Loading assay with UBE2I and SUMO, in the presence of Mg-ATP.
Assay buffer: 50mM HEPES.

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Clone Data Sheet

Protein name GST-SAE1 / His-SAE2

Protein	SAE1 1-364 = full length
Synonyms	AOS1, SUA1, UBLE1A
Clone Number	SC20972
Species	Human
Accession Number	SAE1 Protein: Q9UBE0
Tags	N-terminal GST
GST-SAE1 Amino acid sequence of expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGS**MVEKEEAGGGISEEEAAQYDRQIRLWGLEAQKRLRASRVLLVGLKGLGAEIAKNLILAGVKGLTMLDHEQVTPEDPGAQFLIRTGSVGRNRAEASLERAQNLNPMVDVKVDTEDIEKKPESFFTQFDAVCLTCCSRDVIVKVDQICHKNSIKFFTGDVFGYHGYTFANLGEHEFVEEKTKVAKVSQGVEDGPDTKRAKLDSSETTMVKKKVVFCPVKEALEVDWSSEKAKAALKRTTSDYFLLQVLLKFRTDKGRDPSSDTYEEDSELLLQIRNDVLDSLGISPDLLPEDFVRYCFSEMAPVCAVVGGILAQEIVKALSQRDPPHNNFFFFDGMKGNGIVECLGPK**
Native sequence	SAE1 in **Bold,**
Protease cleavage	Thrombin site underlined
Cloning sites	Kpn1 / Xho1

DNA sequence of SAE1 insert	GGTACCATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGATCTGGTTCCGCGTGGATCCATGGTGGAGAAGGAGGAGGCTGGCGGCGGCATTAGCGAGGAGGAGGCGGCACAGTATGACCGGCAGATCCGCCTGTGGGGACTGGAGGCCCAGAAACGGCTGCGGGCCTCTCGGGTGCTTCTTGTCGGCTTGAAAGGACTTGGGGCTGAAATTGCCAAGAATCTCATCTTGGCAGGAGTGAAAGGACTGACCATGCTGGATCACGAACAGGTAACTCCAGAAGATCCCGGAGCTCAGTTCTTGATTCGTACTGGGTCTGTTGGCCGAAATAGGGCTGAAGCCTCTTTGGAGCGAGCTCAGAATCTCAACCCCATGGTGGATGTGAAGGTGGACACTGAGGATATAGAGAAGAAACCAGAGTCATTTTTCACTCAATTCGATGCTGTGTGTCTGACTTGCTGCTCCAGGGATGTCATAGTTAAAGTTGACCAGATCTGTCACAAAAATAGCATCAAGTTCTTTACAGGAGATGTTTTTGGCTACCATGGATACACATTTGCCAATCTAGGAGAGCATGAGTTTGTAGAGGAGAAAACTAAAGTTGCCAAAGTTAGCCAAGGAGTAGAAGATGGGCCCGACACCAAGAGAGCAAAACTTGATTCTTCTGAGACAACGATGGTCAAAAAGAAGGTGGTCTTCTGCCCTGTTAAAGAAGCCCTGGAGGTGGACTGGAGCAGTGAGAAAGCAAAGGCTGCTCTGAAGCGCACGACCTCCGACTACTTTCTCCTTCAAGTGCTCTTAAAGTTCCGTACAGATAAAGGAAGAGATCCCAGTTCTGATACATATGAGGAAGATTCTGAGTTGTTGCTCCAGATACGAAATGATGTGCTTGACTCACTGGGTATTAGTCCTGACCTGCTTCCTGAGGACTTTGTCAGGTACTGCTTCTCCGAGATGGCCCCAGTGTGTGCGGTGGTTGGAGGGATTTTGGCACAGGAAATTGTGAAGGCCCTGTCTCAGCGGGACCCTCCTCACAACAACTTCTTCTTCTTCGATGGCATGAAGGGGAATGGGATTGTGGAGTGCCTTGGCCCCAAGTGACTCGAG

Protein	SAE2 1-640 = full length
Synonyms	UBA2, Anthracycline-associated resistance ARX
Clone Number	SC20972
Species	Human
Accession Number	SAE2 Protein: Q9UBT2
Tags	N-terminal His6
His-SAE2 Amino acid sequence of expressed protein	PWHHHHHHLEVLFQGP**MALSRGLPRELAEAVAGGRVLVVGAGGIGCELLK** **NLVLTGFSHIDLIDLDTIDVSNLNRQFLFQKKHVGRSKAQVAKESVLQFY** **PKANIVAYHDSIMNPDYNVEFFRQFILVMNALDNRAARNHVNRMCLAADV** **PLIESGTAGYLGQVTTIKKGVTECYECHPKPTQRTFPGCTIRNTPSEPIH** **CIVWAKYLFNQLFGEEDADQEVSPDRADPEAAWEPTEAEARARASNEDGD** **IKRISTKEWAKSTGYDPVKLFTKLFKDDIRYLLTMDKLWRKRKPPVPLDW** **AEVQSQGEETNASDQQNEPQLGLKDQQVLDVKSYARLFSKSIETLRVHLA** **EKGDGAELIWDKDDPSAMDFVTSAANLRMHIFSMNMKSRFDIKSMAGNII** **PAIATTNAVIAGLIVLEGLKILSGKIDQCRTIFLNKQPNPRKKLLVPCAL** **DPPNPNCYVCASKPEVTVRLNVHKVTVLTLQDKIVKEKFAMVAPDVQIED** **GKGTILISSEEGETEANNHKKLSEFGIRNGSRLQADDFLQDYTLLINILH** **SEDLGKDVEFEVVGDAPEKVGPKQAEDAAKSITNGSDDGAQPSTSTAQEQ** **DDVLIVDSDEEDSSNNADVSEEERSRKRKLDEKENLSAKRSRIEQKEELD** **DVIALD**
Native sequence	SAE2 in **Bold,**
Protease cleavage	Prescission site underlined
Cloning sites	Nco1 / Sal1

DNA sequence of SAE2 insert	CCATGGGACACCATCACCATCACCATCTGGAAGTTCTGTTCCAGGGGCCA ATGGCACTGTCGCGGGGGCTGCCCCGGGAGCTGGCTGAGGCGGTGGCCGG GGGCCGGGTGCTGGTGGTGGGGGCGGGCGGCATCGGCTGCGAGCTCCTCA AGAATCTCGTGCTCACCGGTTTCTCCCACATCGACCTGATTGATCTGGAT ACTATTGATGTAAGCAACCTCAACAGACAGTTTTTGTTTCAAAAGAAACA TGTTGGAAGATCAAAGGCACAGGTTGCCAAGGAAAGTGTACTGCAGTTTT ACCCGAAAGCTAATATCGTTGCCTACCATGACAGCATCATGAACCCTGAC TATAATGTGGAATTTTTCCGACAGTTTATACTGGTTATGAATGCTTTAGA TAACAGAGCTGCCCGAAACCATGTTAATAGAATGTGCCTGGCAGCTGATG TTCCTCTTATTGAAAGTGGAACAGCTGGGTATCTTGGACAAGTAACTACT ATCAAAAAGGGTGTGACCGAGTGTTATGAGTGTCATCCTAAGCCGACCCA GAGAACCTTTCCTGGCTGTACAATTCGTAACACACCTTCAGAACCTATAC ATTGCATCGTTTGGGCAAAGTACTTGTTCAACCAGTTGTTTGGGGAAGAA GATGCTGATCAAGAAGTATCTCCTGACAGAGCTGACCCTGAAGCTGCCTG GGAACCAACGGAAGCCGAAGCCAGAGCTAGAGCATCTAATGAAGATGGTG ACATTAAACGTATTTCTACTAAGGAATGGGCTAAATCAACTGGATATGAT CCAGTTAAACTTTTTACCAAGCTTTTTAAAGATGACATCAGGTATCTGTT GACAATGGACAAACTATGGCGGAAAAGGAAACCTCCAGTTCCGTTGGACT GGGCTGAAGTACAAAGTCAAGGAGAAGAAACGAATGCATCAGATCAACAG AATGAACCCCAGTTAGGCCTGAAAGACCAGCAGGTTCTAGATGTAAAGAG CTATGCACGTCTTTTTTCAAAGAGCATCGAGACTTTGAGAGTTCATTTAG CAGAAAAGGGGGATGGAGCTGAGCTCATATGGGATAAGGATGACCCATCT

	GCAATGGATTTTGTCACCTCTGCTGCAAACCTCAGGATGCATATTTTCAGTATGAATATGAAGAGTAGATTTGATATCAAATCAATGGCAGGGAACATTATTCCTGCTATTGCTACTACTAATGCAGTAATTGCTGGGTTGATAGTATTGGAAGGATTGAAGATTTTATCAGGAAAAATAGACCAGTGCAGAACAATTTTTTTGAATAAACAACCAAACCCAAGAAAGAAGCTTCTTGTGCCTTGTGCACTGGATCCTCCCAACCCCAATTGTTATGTATGTGCCAGCAAGCCAGAGGTGACTGTGCGGCTGAATGTCCATAAAGTGACTGTTCTCACCTTACAAGACAAGATAGTGAAAGAAAAATTTGCTATGGTAGCACCAGATGTCCAAATTGAAGATGGGAAAGGAACAATCCTAATATCTTCCGAAGAGGGAGAGACGGAAGCTAATAATCACAAGAAGTTGTCAGAATTTGGAATTAGAAATGGCAGCCGGCTTCAAGCAGATGACTTCCTCCAGGACTATACTTTATTGATCAACATCCTTCATAGTGAAGACCTAGGAAAGGACGTTGAATTTGAAGTTGTTGGTGATGCCCCGGAAAAAGTGGGGCCCAAACAAGCTGAAGATGCTGCCAAAAGCATAACCAATGGCAGTGATGATGGAGCTCAGCCCTCCACCTCCACAGCTCAAGAGCAAGATGACGTTCTCATAGTTGATTCAGATGAAGAAGATTCTTCAAATAATGCCGACGTCAGTGAAGAAGAGAGAAGCCGCAAGAGGAAATTAGATGAGAAAGAGAATCTCAGTGCAAAGAGGTCACGTATAGAACAGAAGGAAGAGCTTGATGATGTCATAGCATTAGATTGAGTCGAC