

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of FKHR [329 - 655]

Protein description:- FKHR [329 - 655]

Clone number:- DU 1071

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 8 mg/L

Calculated molecular mass:- 61, 974 daltons

Purity:- 90 %

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

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CLONE DATA SHEET

FKHR [329 - 655]

<u>Protein</u>	FKHR [329 - 655]
<u>Clone number</u>	DU 1071
<u>Species</u>	Human
<u>Accession number</u>	NM_002015
<u>Tags</u>	N-terminal GST
<u>Bacterially expressed protein</u>	MSPILGYWKIKGLVQPTRLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNLGGCPKERAIEISMLE GAVLDIRYGVSR IAYS KDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAI PQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVL FQGPLGSIMTEQDDL G EGDVHSMVYPPSAAKMASTLPSLSEISNPENMENLLDNLNLLSSPTSL TVSTQSSPGTMMQQTPCYSFAPPNTSLNSPSPNYQKYTYGQSSMSPLP QMPIQTLQDNKSSYGGMSQYNCAPGLLKELLTSDSPPHNDIMTPVDPG VAQPNSRVLGQNVMMGPNSVMSTYGSQASHNKMMNPSSHTHPGHAQQT SAVNGRPLPHTVSTMPHTSGMNRLTQVKTPVQVPLPHPMQMSALGGYS SVSSCNGYGRMGLLHQEKLPDLDGMFIERLDCDMESIIRNDLMDGDT LDFNFDNVLPNQSFPHSVKTTTHSWVSG
<u>Native sequence</u>	Amino acids I329 – G655 (end) of human FKHR. Residue M232 of the fusion protein is equivalent to I329 of the native protein. The GST tag is located at residues 1 - 220
<u>Protease cleavage</u>	PreScission (<u>LEVLFQGPL</u>) residues 221 - 229
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Sal</i> I of pGEX-6P-1

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Nucleotide sequence

GGATCCATTATGACCGAACAGGATGATCTTGGAGAAGGGGA
TGTGCATTCTATGGTGTACCCGCCATCTGCCGCAAAGATGG
CCTCTACTTTACCCAGTCTGTCTGAGATAAGCAATCCCGAA
AACATGGAAAATCTTTTGGATAATCTCAACCTTCTCTCATC
ACCAACATCATTAAGTGTTCGACCCAGTCCTCACCTGGCA
CCATGATGCAGCAGACGCCGTGCTACTCGTTTGCGCCACCA
AACACCAGTTTGAATTACCCAGCCCAAACCTACCAAAAATA
TACATATGGCCAATCCAGCATGAGCCCTTTGCCCCAGATGC
CTATACAAACACTTCAGGACAATAAGTCGAGTTATGGAGGT
ATGAGTCAGTATAACTGTGCGCCTGGACTCTTGAAGGAGTT
GCTGACTTCTGACTCTCCTCCCCATAATGACATTATGACAC
CAGTTGATCCTGGGGTAGCCCAGCCCAACAGCCGGGTTCTG
GGCCAGAACGTCATGATGGGCCCTAATTCGGTCATGTCAAC
CTATGGCAGCCAGGCATCTCATAACAAAATGATGAATCCCA
GCTCCCATACCCACCCTGGACATGCTCAGCAGACATCTGCA
GTTAACGGGCGTCCCCTGCCCCACACGGTAAGCACCATGCC
CCACACCTCGGGTATGAACCGCCTGACCCAAGTGAAGACAC
CTGTACAAGTGCCCTCTGCCCCACCCCATGCAGATGAGTGCC
CTGGGGGGCTACTCCTCCGTGAGCAGCTGCAATGGCTATGG
CAGAATGGGCCTTCTCCACCAGGAGAAGCTCCCAAGTGACT
TGGATGGCATGTTTCATTGAGCGCTTAGACTGTGACATGGAA
TCCATCATTCGGAATGACCTCATGGATGGAGATACATTGGA
TTTTAACTTTGACAATGTGTTGCCCAACCAAAGCTTCCCAC
ACAGTGTCAAGACAACGACACATAGCTGGGTGTCAGGctga
gtcgac