Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of FKHR [2 - 655]

Protein description:- FKHR [2 - 655]

Clone number:- DU 1725

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST and C-terminal His(6)

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

Expression level:- 1 mg/L

Calculated molecular mass:- 97, 115 daltons

Purity:- 85 %

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 [2 - 655]

Protein FKHR [2 - 655]

Clone number DU 1725

Species Human

Accession number NM_002015

Tags N-terminal GST and C-terminal His(6)

Bacterially expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**AEAPQVVEI**
DPDFEPLPRPRSCTWPLPRPEFSQSNSATSSPAPSGSAAANPDAAAGL
PSASAAAVSADFMSNLSLLEESEDFPQAPGSVAAAVAAAAAAAATGGL
CGDFQGPEAGCLHPAPPQPPPPGPLSQHPPVPPAAAGPLAGQPRKSSS
SRRNAWGNLSYADLITKAIESSAEKRLTLSQIYEWMVKSVPYFKDKGD
SNSSAGWKNSIRHNLSLHSKFIRVQNEGTGKSSWWMLNPEGGKSGKSP
RRRAASMDNNSKFAKSRSRAAKKKASLQSGQEGAGDSPGSQFSKWPAS
PGSHSNDDFDNWSTFRPRTSSNASTISGRLSPIMTEQDDLGEGDVHSM
VYPPSAAKMASTLPSLSEISNPENMENLLDNLNLLSSPTSLTVSTQSS
PGTMMQQTPCYSFAPPNTSLNSPSPNYQKYTYGQSSMSPLPQMPIQTL
QDNKSSYGGMSQYNCAPGLLKELLTSDSPPHNDIMTPVDPGVAQPNSR
VLGQNVMMGPNSVMSTYGSQASHNKMMNPSSHTHPGHAQQTSAVNGRP
LPHTVSTMPHTSGMNRLTQVKTPVQVPLPHPMQMSALGGYSSVSSCNG
YGRMGLLHQEKLPSDLDGMFIERLDCDMESIIRNDLMDGDTLDFNFDN
VLPNQSFPHSVKTTTHSWVSGHHHHHH

Native sequence Amino acids A2 – G655 (end) of human FKHR.
Residues A232 of the fusion protein is equilivalent to A2 of the native protein. The GST tag is located at residues 1 – 220 and the His(6) tag is located at residues 886 – 891.

Protease cleavage PreScission (LEVLFQGPL) residues 221 - 229

Cloning sites *Bam*HI and *Sal*I sites of pGEX 6P-1

Nucleotide sequence	Re-sequencing the clone at present