MRCPPU Reagents and Services

Standard Operating Procedure

Preparation of active BTK C481S [2 – 659]

Enzyme description:- NTK C481S [2 - 659]

Clone number:- DU 79155

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His6

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

Calculated molecular mass:-
Monoisotopic 79, 454.75 daltons
Average Mass 79, 505.62 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 7.06

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, 0.5 mM TCEP

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 5 mM MgAc

Substrate:-
KVEKIGEGTYGVVYK Final concentration: 300 uM

Clone Data Sheet

BTK C481S [2 – 659]

Protein	BTK C481S [2 – 659]
Clone number	DU 79155
Species	Human
Accession number	NP_00052.1
Tags	N-terminal HIS6

Baculovirus expressed protein

MSYYHHHHHHDYDIPTTENLYFQGAMGS**AAVILESIFLKRSQQK**
KKTSPLNFKKRLFLLTVHKLSYYEYDFERGRRGSKKGSIDVEKI
TCVETVVPEKNPPPERQIPRRGEESSEMEQISIIERFPYPFQVV
YDEGPLYVFSPTEELRKRWIHQLKNVIRYNSDLVQKYHPCFWID
GQYLCCSQTAKNAMGCQILENRNGSLKPGSSHRKTKKPLPPTPE
EDQILKKPLPPEPAAAPVSTSELKKVVALYDYMPMNANDLQLRK
GDEYFILEESNLPWWRARDKNGQEGYIPSNYVTEAEDSIEMYEW
YSKHMTRSQAEQLLKQEGKEGGFIVRDSSKAGKYTVSVFAKSTG
DPQGVIRHYVVCSTPQSQYYLAEKHLFSTIPELINYHQHNSAGL
ISRLKYPVSQQNKNAPSTAGLGYGSWEIDPKDLTFLKELGTGQF
GVVKYGKWRGQYDVAIKMIKEGSMSEDEFIEEAKVMMNLSHEKL
VQLYGVCTKQRPIFIITEYMANGSLLNYLREMRHRFQTQQLLEM
CKDVCEAMEYLESKQFLHRDLAARNCLVNDQGVVKVSDFGLSRY
VLDDEYTSSVGSKFPVRWSPPEVLMYSKFSSKSDIWAFGVLMWE
IYSLGKMPYERFTNSETAEHIAQGLRLYRPHLASEKVYTIMYSC
WHEKADERPTFKILLSNILDVMDEES

Native sequence

Amino acids A2 – S659 (end residue) of human BTK.
Residue A29 of the fusion protein is equivalent to A2 of the native enzyme. The His(6) tag is located at residues 5 - 10.

The enzyme has a C481S mutation that confirms drug resistance. Residue C481 is equivalent to S508 of the fusion protein

Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Bam*H1 and *Not*1sites of pFastBac Htb