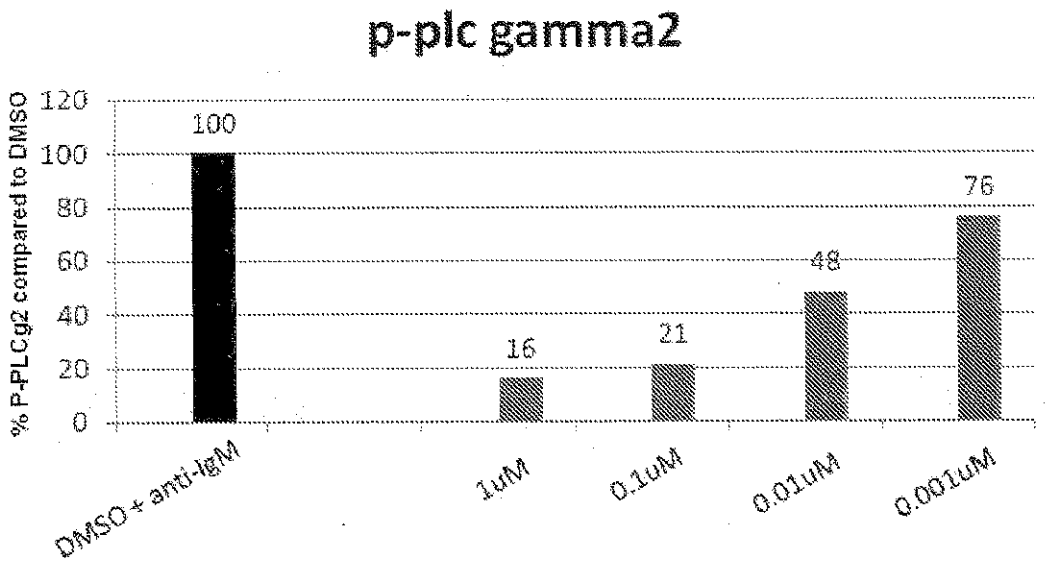
Dose-Response with Compound **I-2** in Ramos cells



Washout with 1 µM Compound **I-2** in Ramos cells

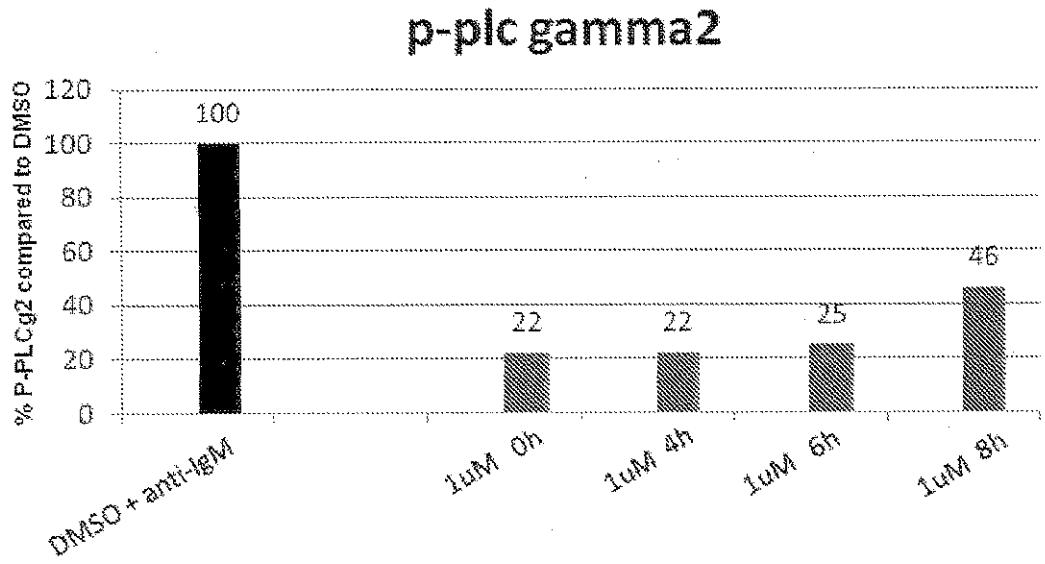
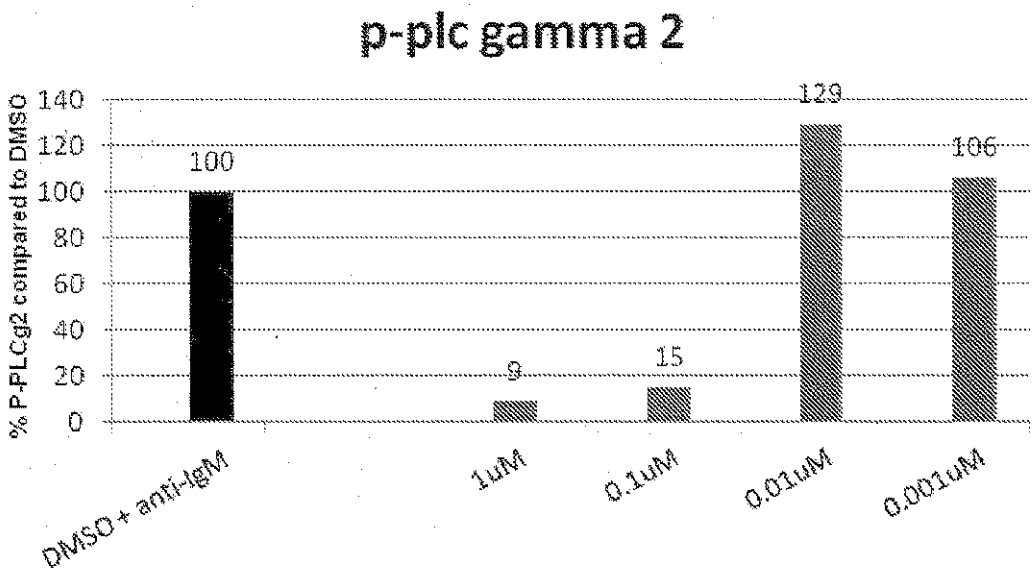


Figure 1

Dose-Response with Compound **I-4** in Ramos cells



Washout with 1 µM Compound **I-4** in Ramos cells

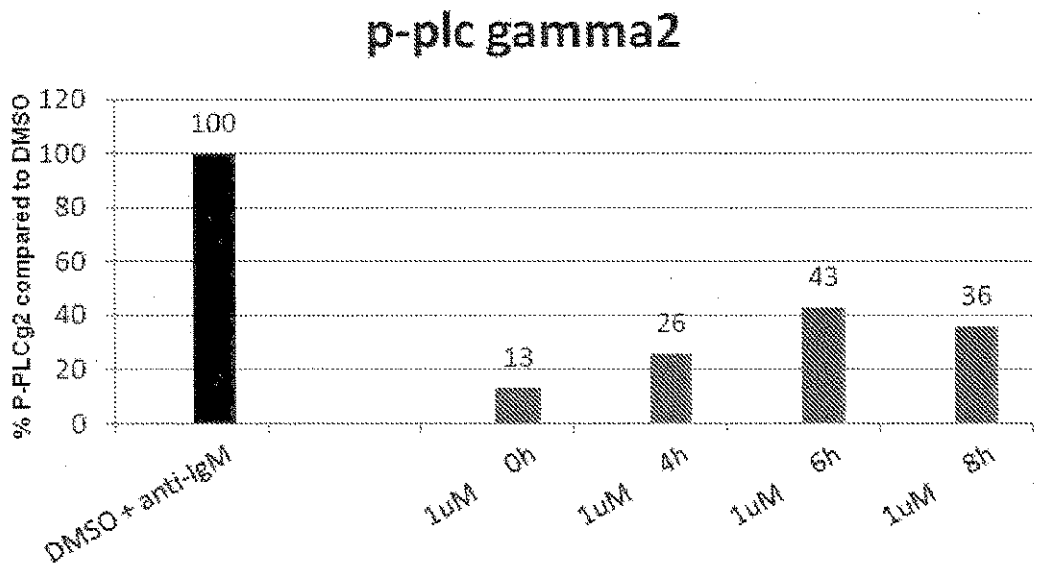
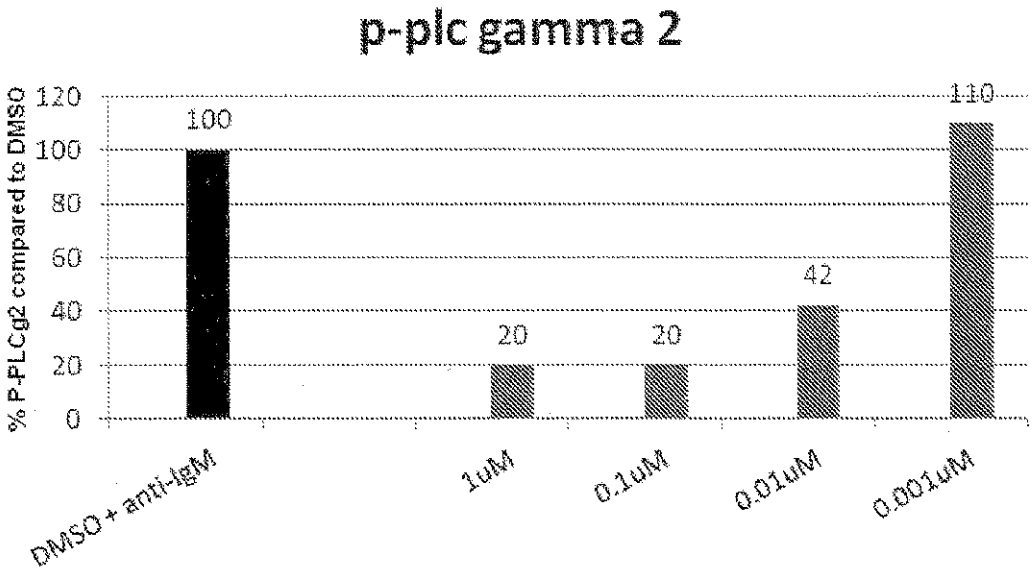


Figure 2

Dose-Response with Compound **I-7** in Ramos cells



Washout Experiment with 1 µM Compound **I-7** in Ramos Cells

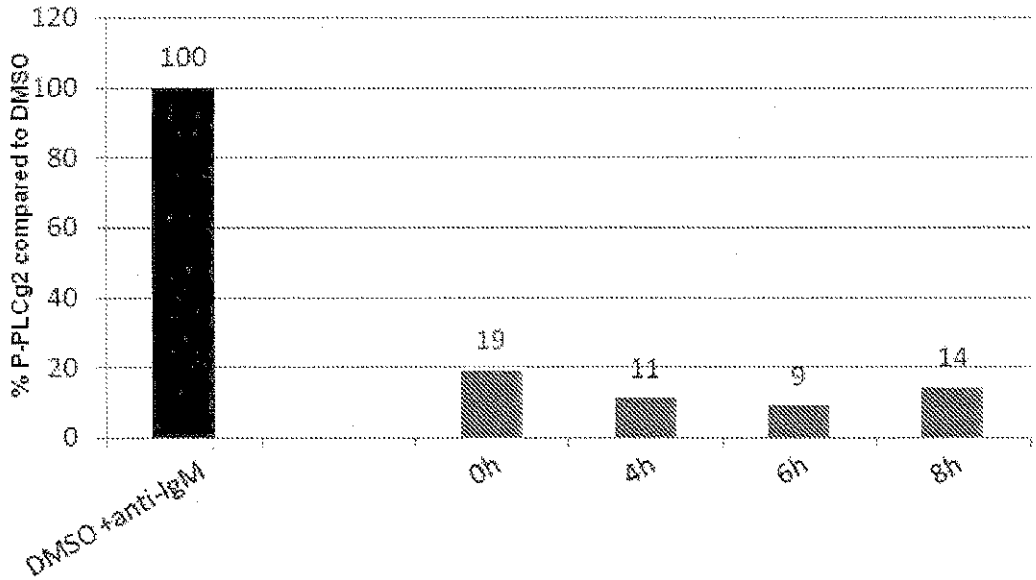


Figure 3

Dose-Response with Compound **I-35** in Ramos cells

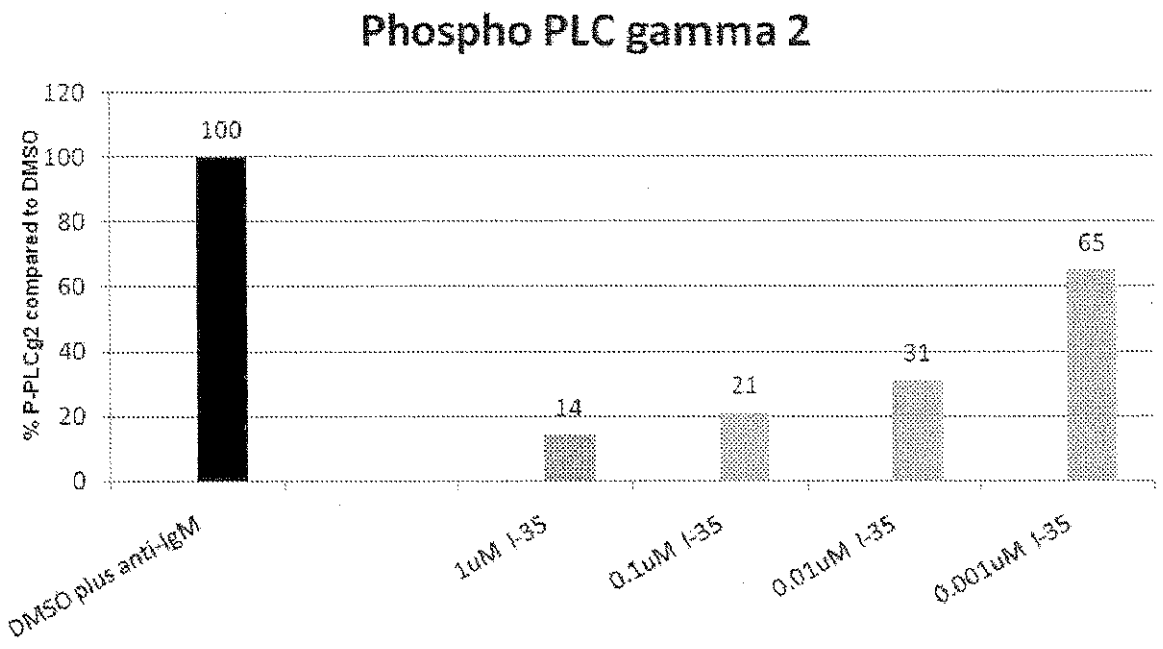


Figure 4

Dose-Response with Compound **I-38** in Ramos cells

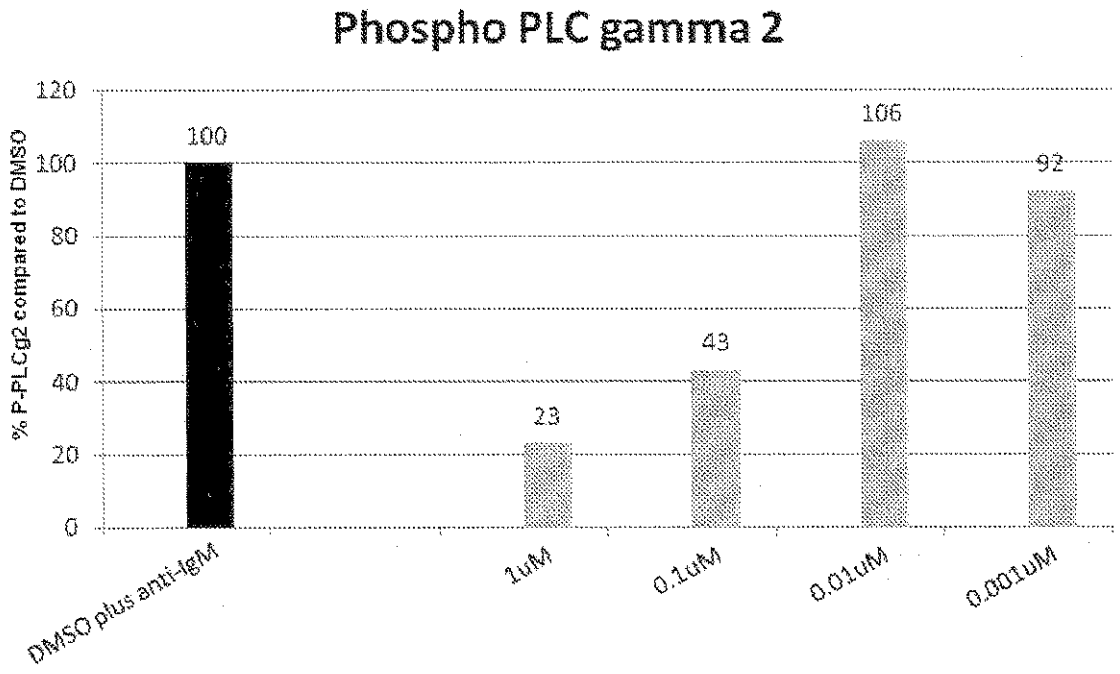


Figure 5

TEC Kinase - Compound **I-2** Tryptic Digest Results

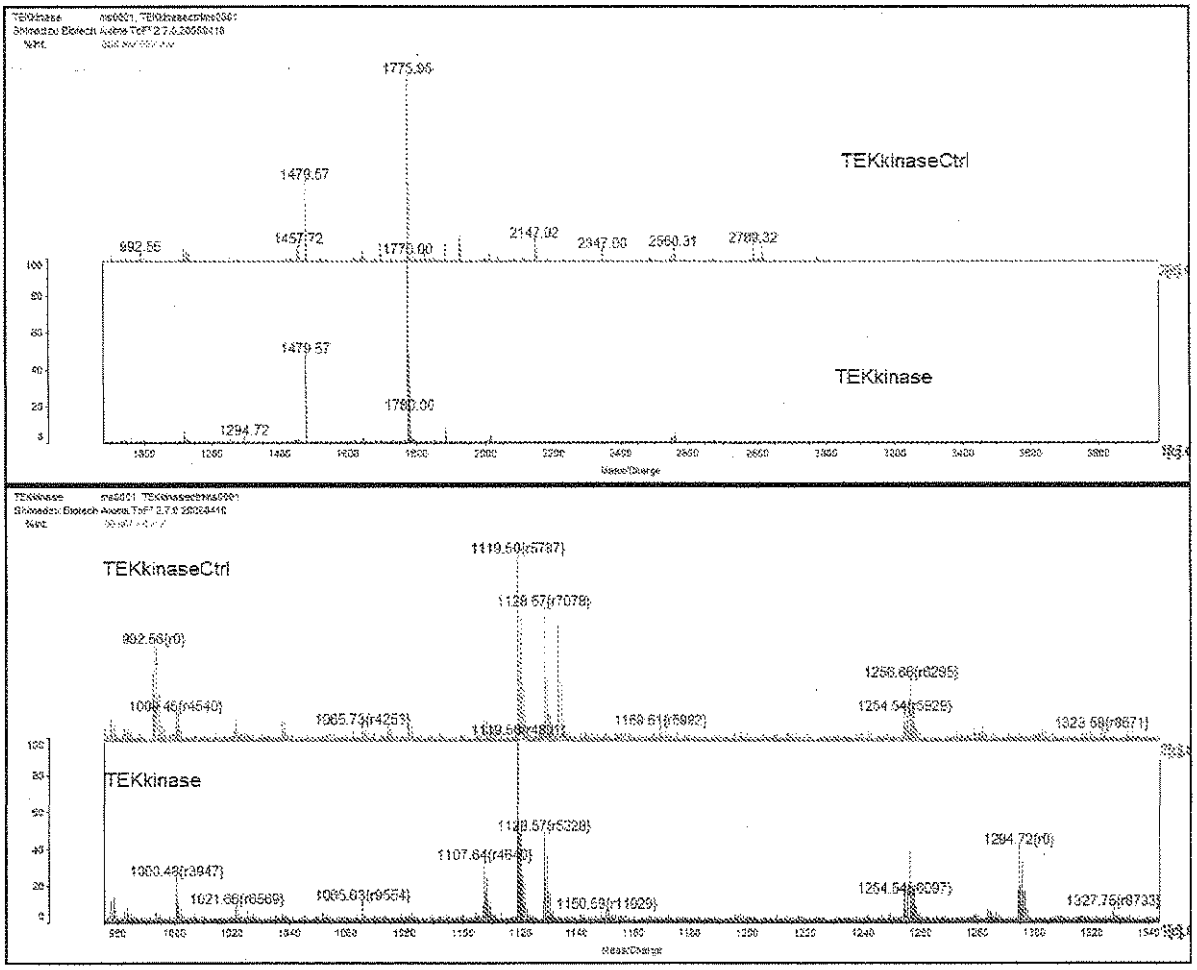


Figure 6

TEC Kinase - Compound **I-4** Tryptic Digest Results

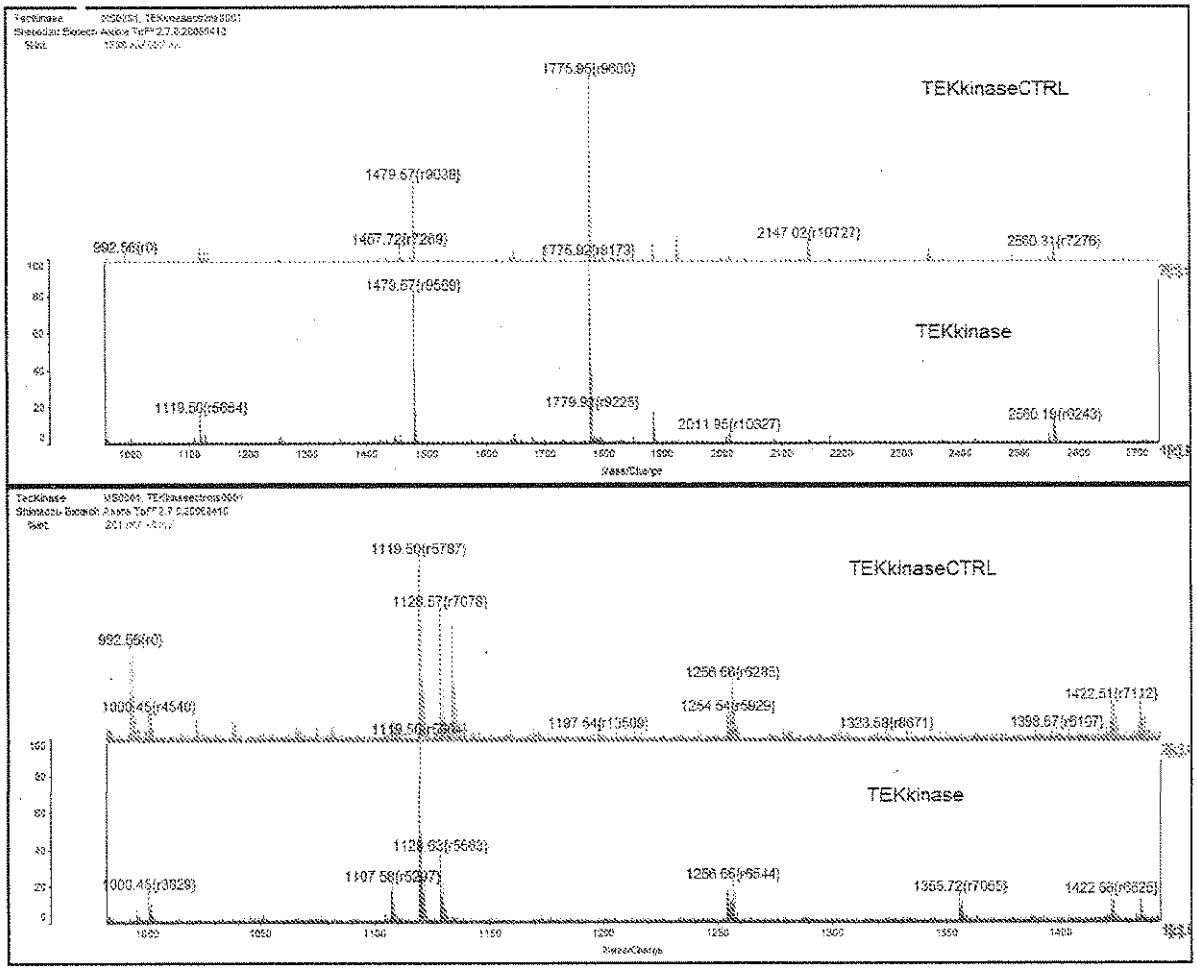


Figure 7

TEC Kinase - Compound **I-7** Tryptic Digest Results

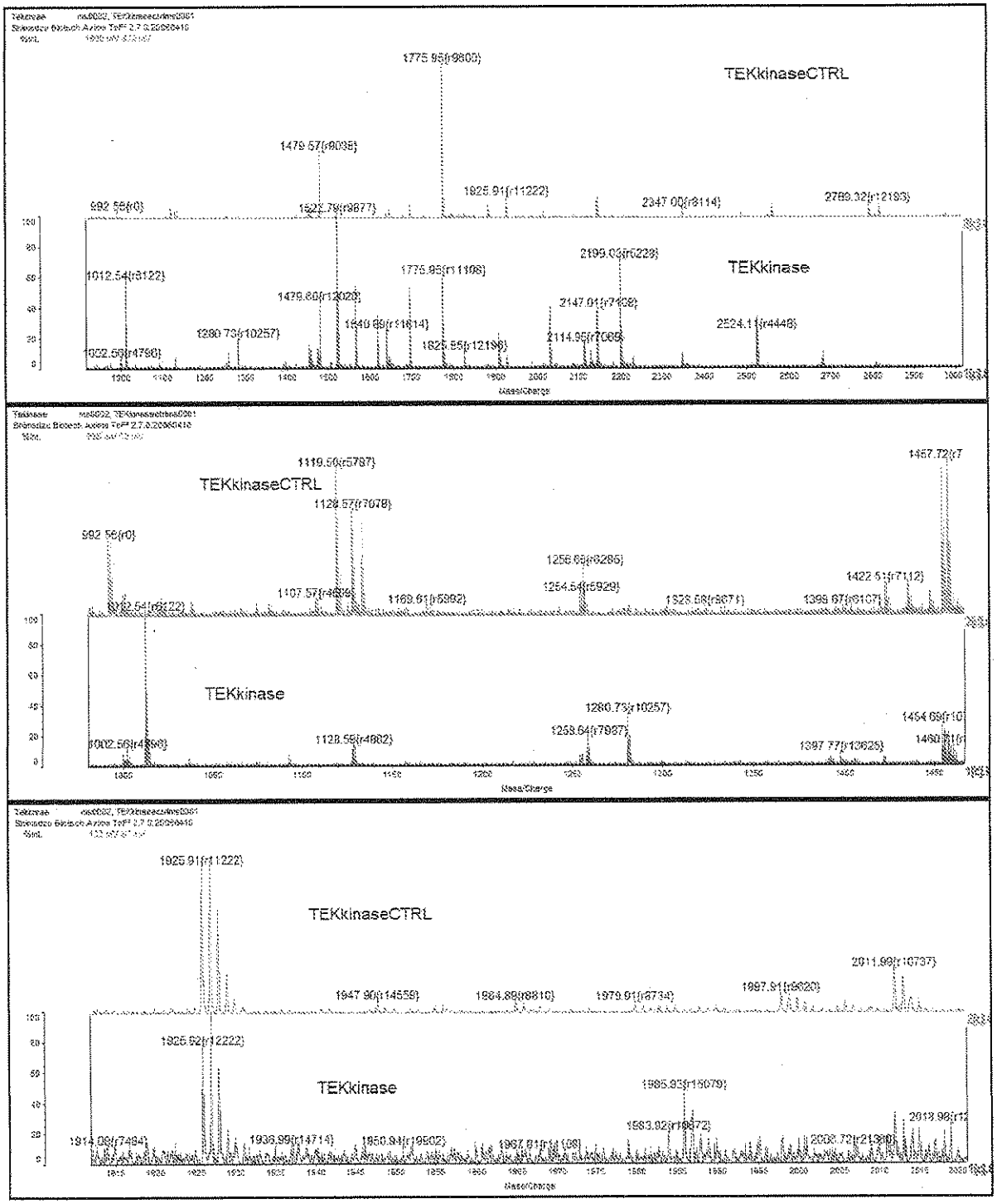
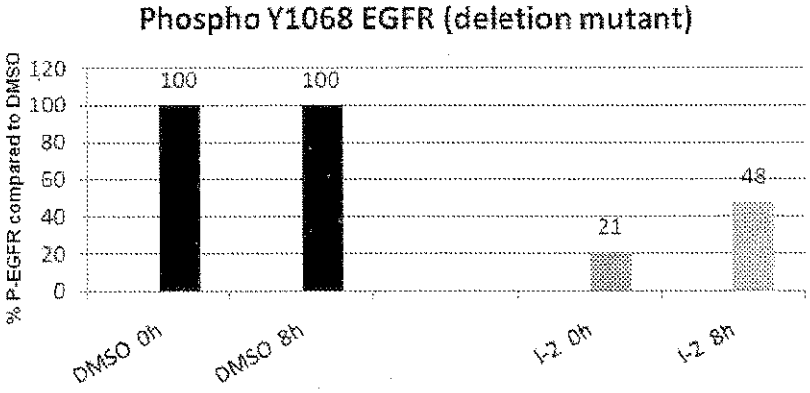
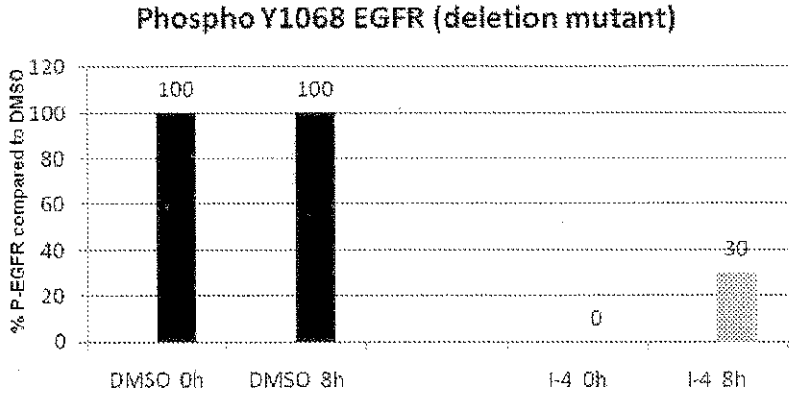


Figure 8

Washout Experiments with Compounds **I-2**, **I-4**, and **I-7** in HCC827 cells containing EGFR Deletion mutant

**Concentration of compound used is 1 μM in all cases





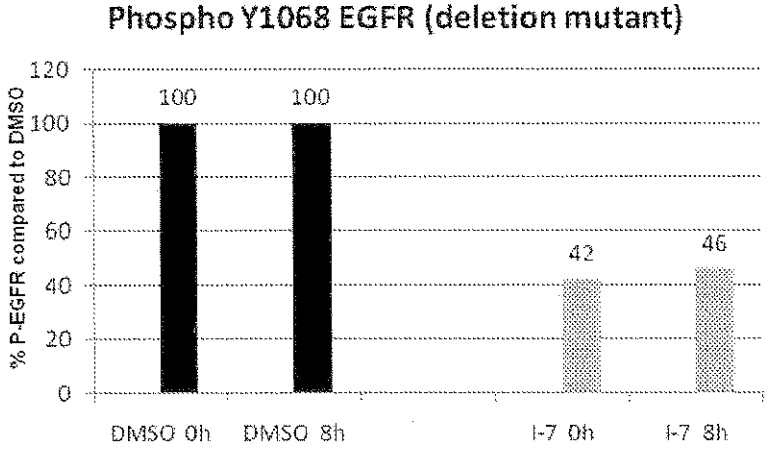


Figure 9

Washout Experiment with 1 μM Compound **I-7** in A431 Cells (EGFR wt)

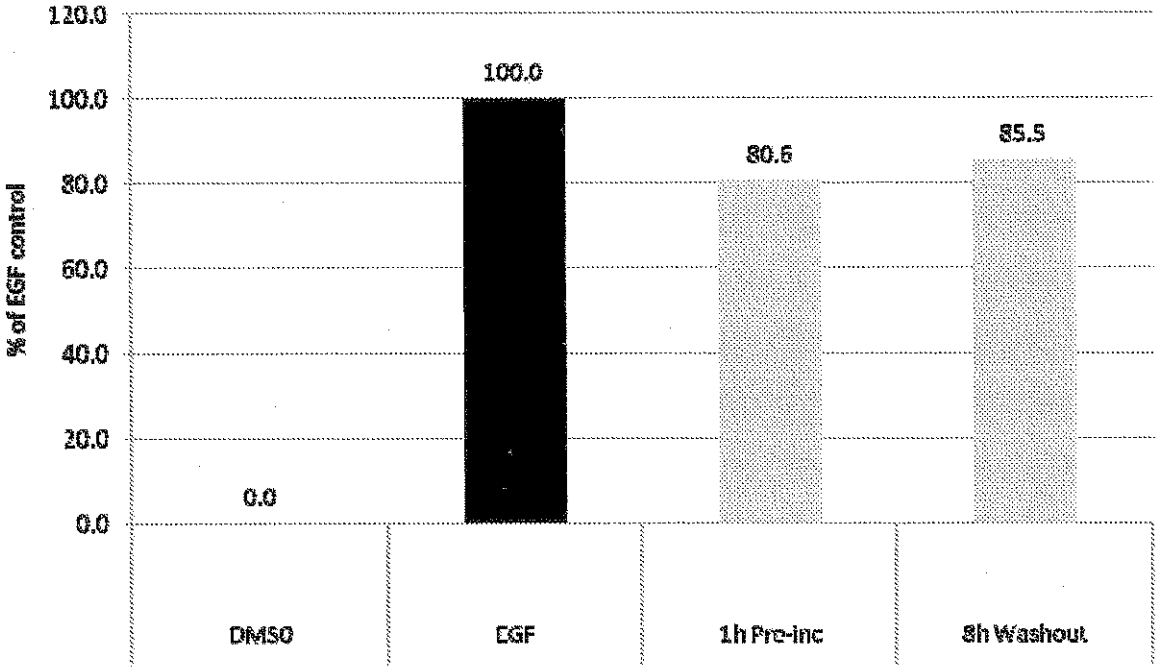


Figure 10

JAK3 Kinase – Compound **I-7** Tryptic Digest Results

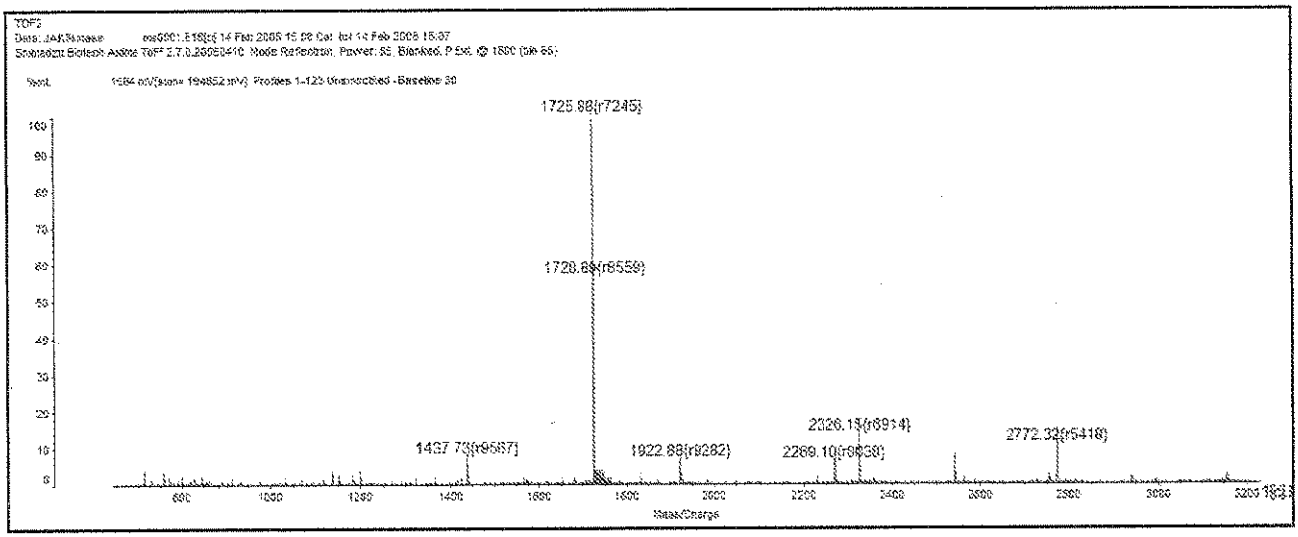


Figure 11

Dose Response with Compound **I-2** in CTLL-2 cells

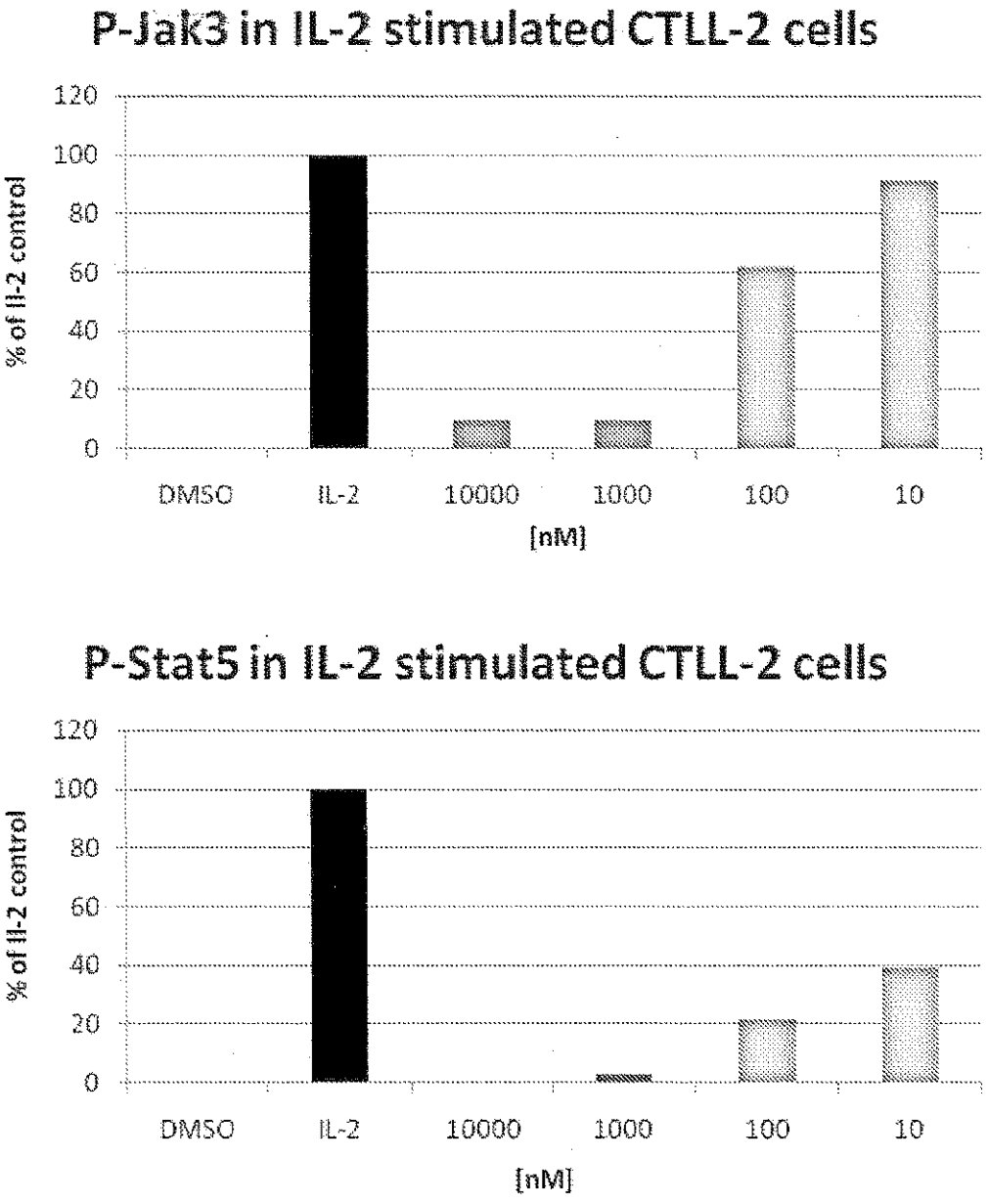
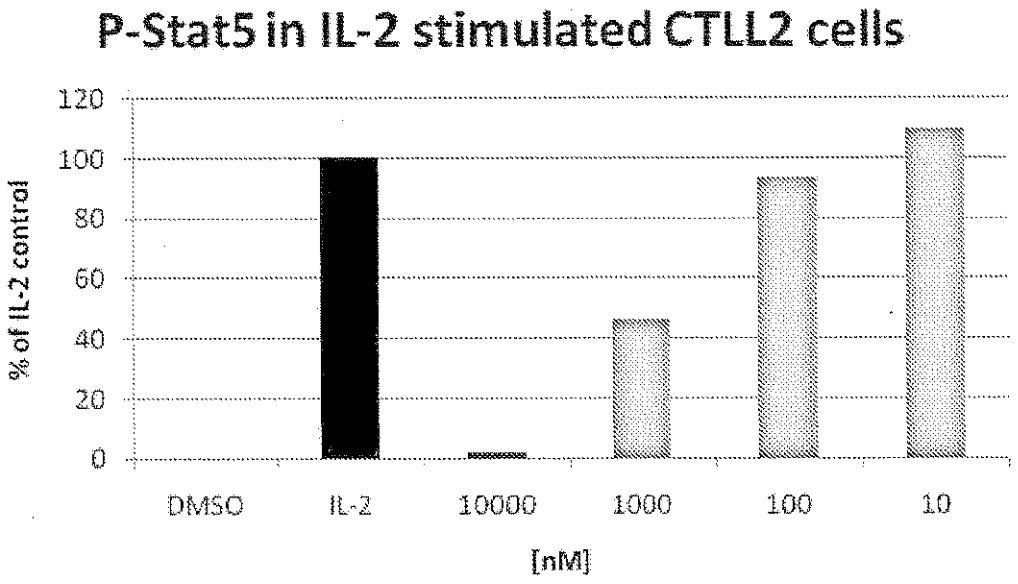


Figure 12

Dose Response with Compound **I-4** in CTLL-2 Cells



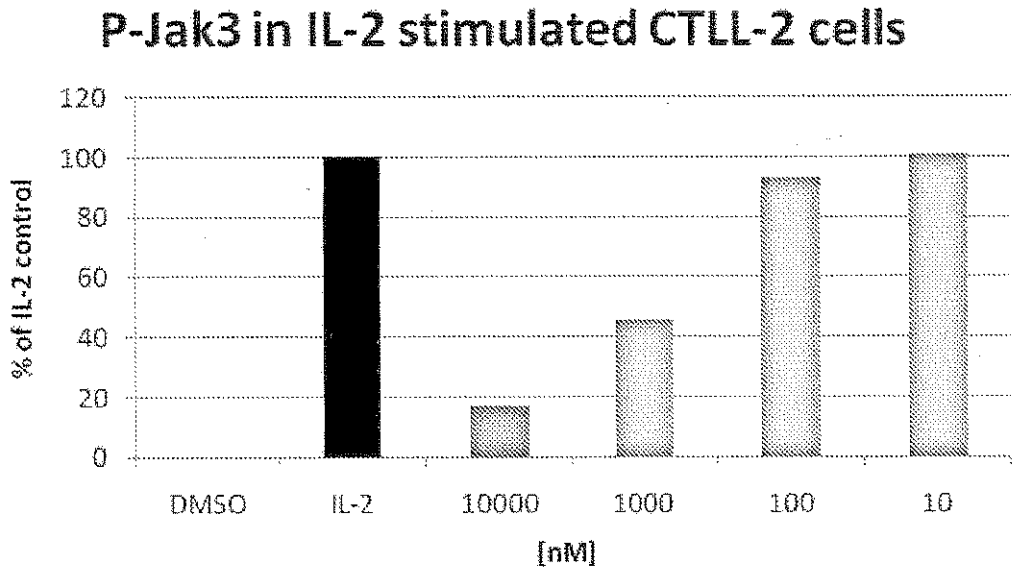


Figure 13

Dose Response with Compound **I-7** in CTLL-2 Cells

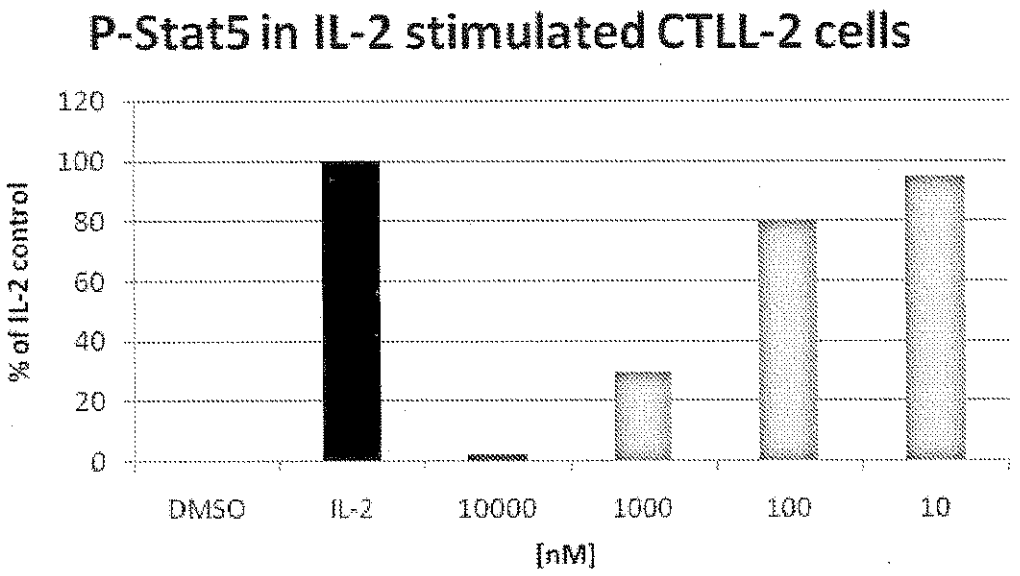


Figure 14

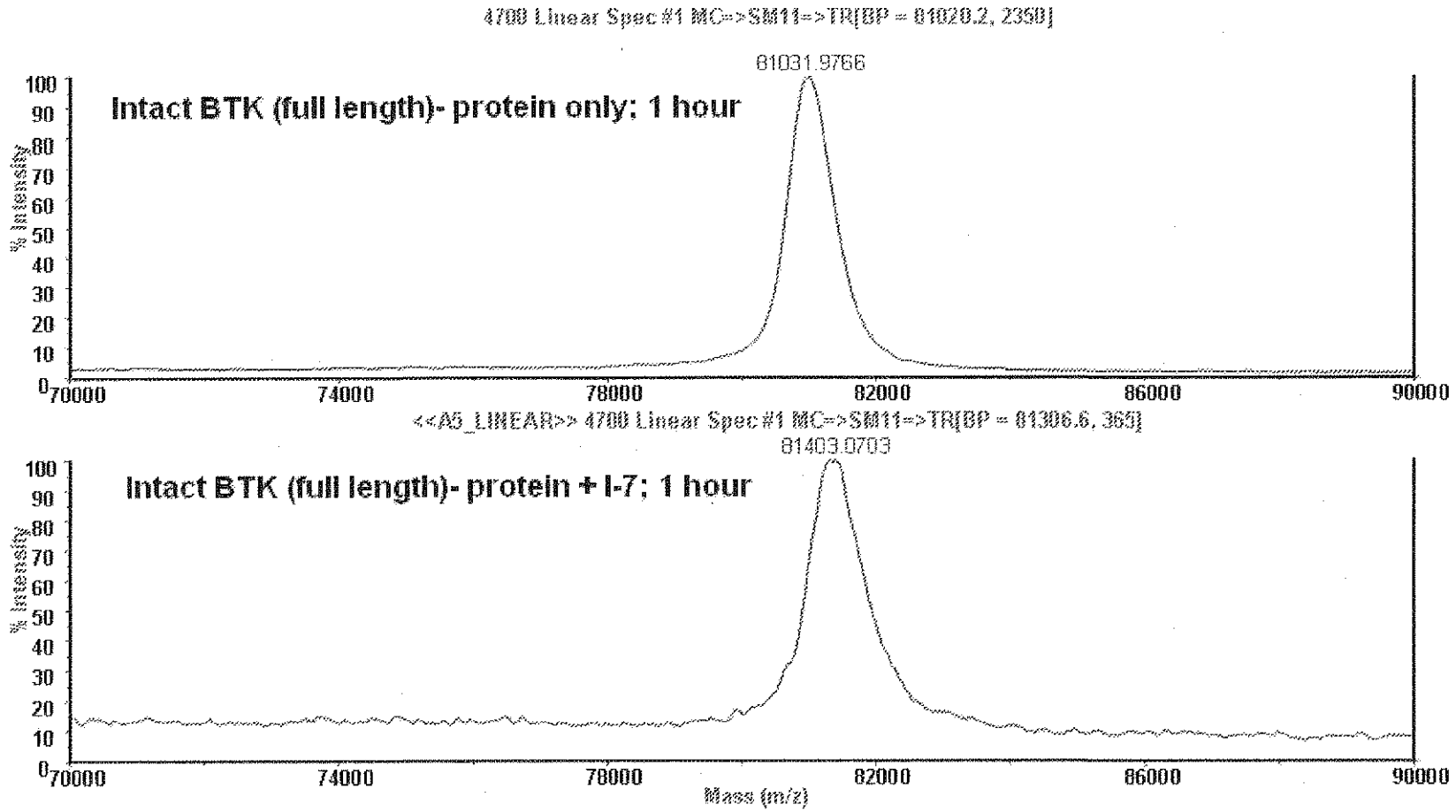

4700 Linear Spec #1 MC=>SM11=>TR[BP = 81020.2, 2350]
81031.9766
Intact BTK (full length)- protein only: 1 hour
% Intensity
100
90
80
70
60
50
40
30
20
10
0
70000
74000
78000
82000
86000
90000
<<A5_LINEAR>> 4700 Linear Spec #1 MC=>SM11=>TR[BP = 81306.6, 365]
81403.0703
Intact BTK (full length)- protein + I-7; 1 hour
% Intensity
Mass (m/z)

Figure 15

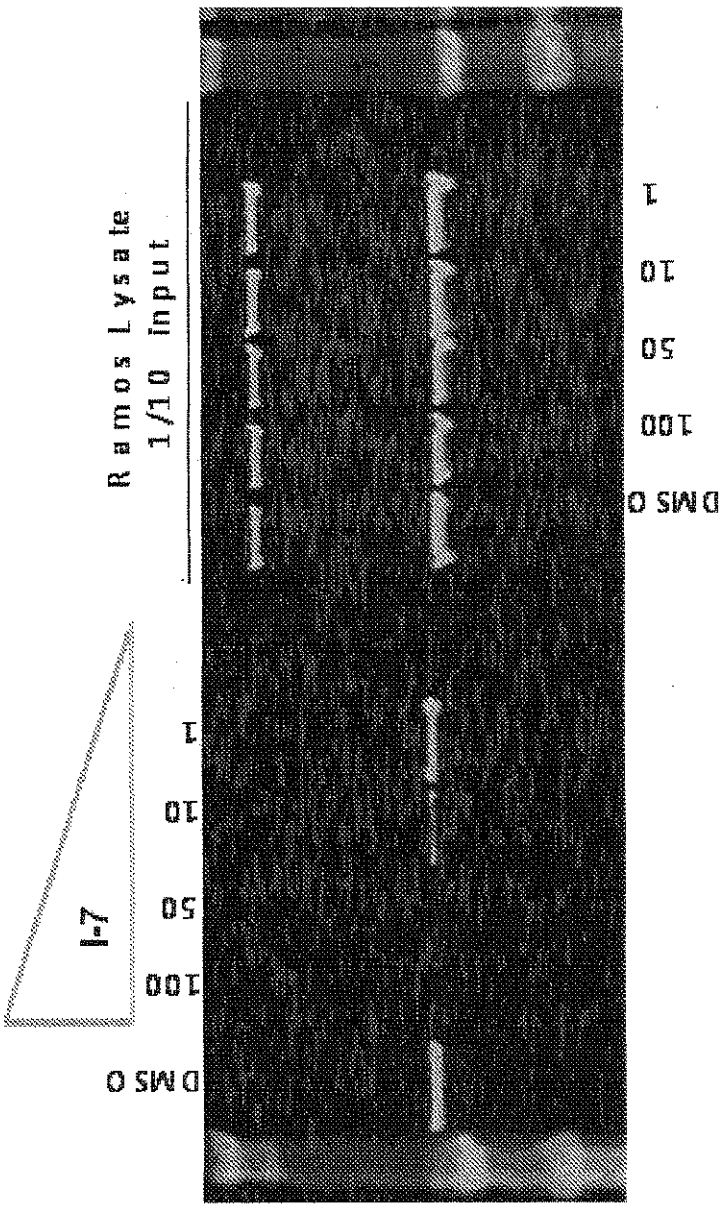


Figure 16

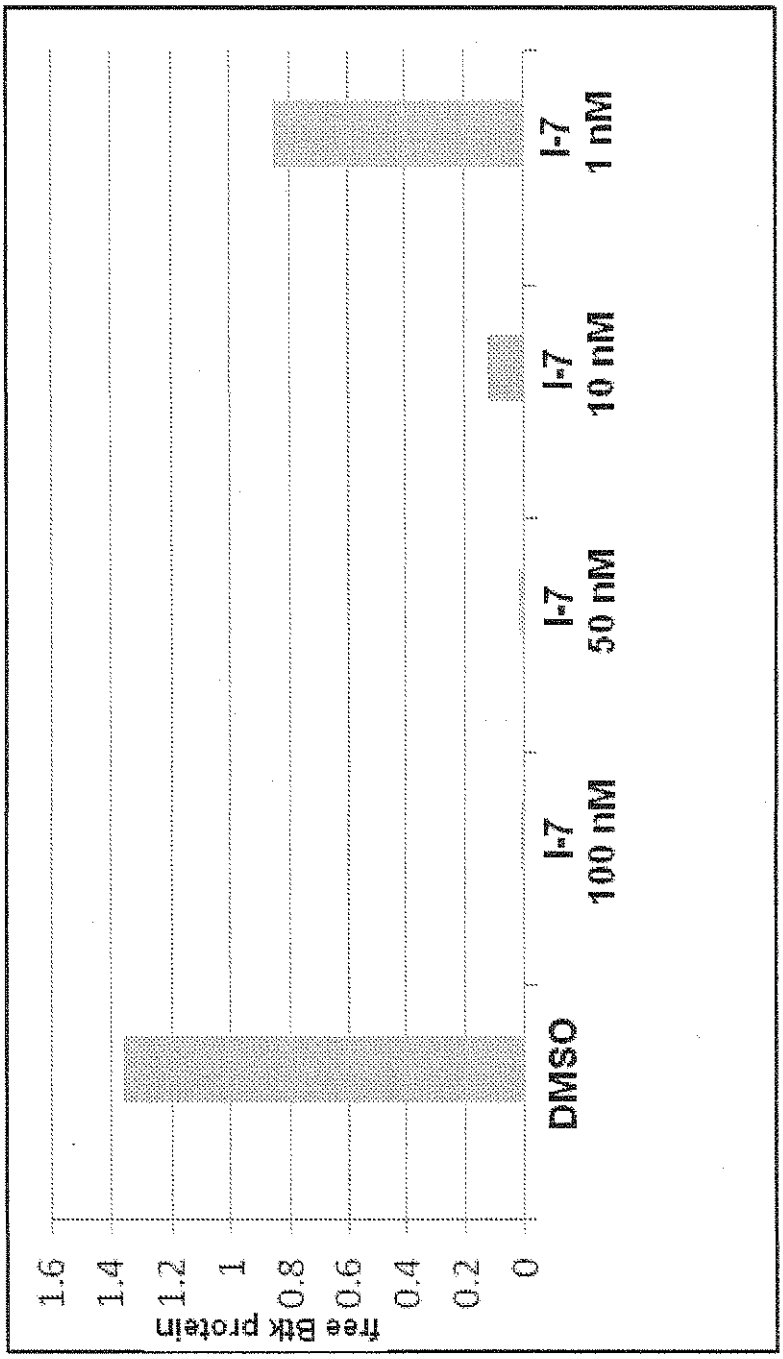

free Btk protein
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
I-7 100 nM
I-7 50 nM
I-7 10 nM
I-7 1 nM

Figure 17

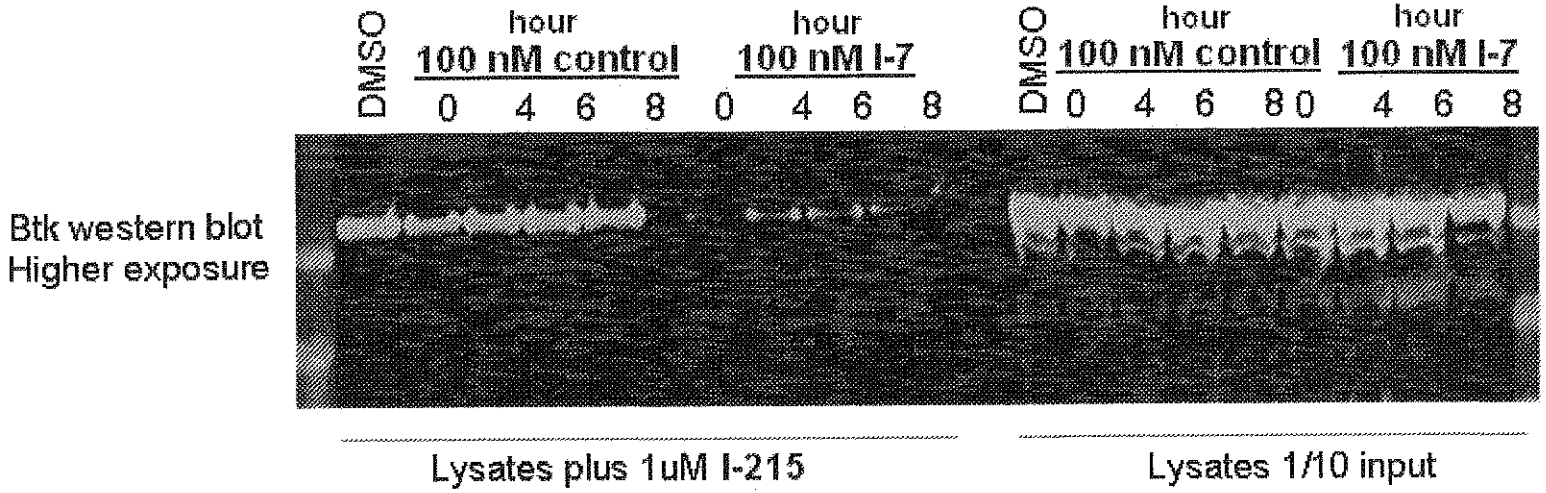

DMSO
hour
100 nM control
0 4 6 8
hour
100 nM I-7
0 4 6 8
DMSO
hour
100 nM control
0 4 6 8
hour
100 nM I-7
0 4 6 8
Btk western blot
Higher exposure
Lysates plus 1uM I-215
Lysates 1/10 input

Figure 18

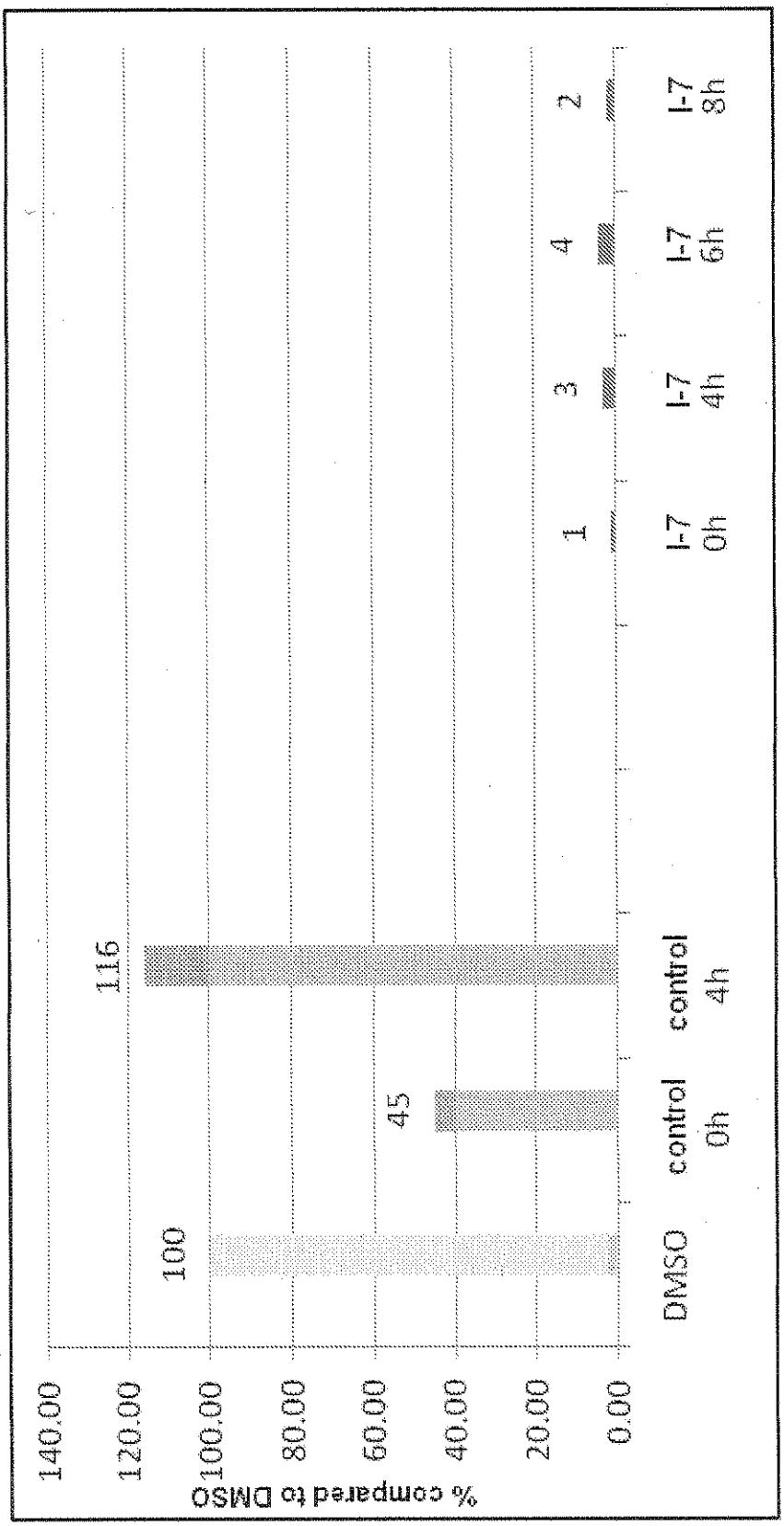

% compared to DMSO
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
116
100
45
DMSO
control 0h
control 4h
1
I-7 0h
3
I-7 4h
4
I-7 6h
2
I-7 8h

Figure 19

SEQ ID 1: FULL LENGTH BTK PROTEIN:

MAAVILESIFLKRSQQKKKTSPLNFKKRLFLLTVHKLSYYEYDFERGRRGSKKGSIDVEK
ITCVETVVPEKNPPPERQIPRRGEESSEMEQISIIERFPYPFQVVYDEGPLYVFSPTEEL
RKRWIHQLKNVIRYNSDLVQKYHPCFWIDGQYLCCSQTAKNAMGCQILENRNGSLKPGSSHRKTKKPLPPTPEEDQILKKPL
PPEPAAAPVSTSELKKVVALYDYMPMNANDLQLRKGDEYFILEESNLPWWRARDKNGQEGYIPSNYVTEAEDSIEMYEWY
SKHMTRSQAEQLLKQEGKEGGFIVRDSSKAGKYTVSVFAKSTGDPQGVIRHYVVCSTPQSQYYLAEKHLFSTIPELINYHQH
NSAGLISRLKYPVSQQNKNAPSTAGLGYGSWEIDPKDLTFLKELGTGQFGVVKYGKWRGQYDVAIKMIKEGSMSEDEFIEEA
KVMMNLSHEKLVQLYGVCTKQRPIFIITEYMANGCLLNYLREMRHRFQTQQLLEMCKDVCEAMEYLESKQFLHRDLAARN
CLVNDQGVVKVSDF

Cys=481

Figure 20

SEQ ID 2: FULL LENGTH TEC PROTEIN NP_003206 631 aa

MNFNTILEEILIKRSQQKKKTSPLNYKERLFVLTKSMLTYYEGRAEKKYRKGFIDVSKIKCVEIVKNDDGVIPCQNKYPFQVV
HDANTLYIFAPSPQSRDLWVKKLKEEIKNNNNIMIKYHPKFWTDGSYQCCRQTEKLAPGCEKYNLFESSIRKALPPAPETKKR
RPPPPIPLEEEDNSEEIVVAMYDFQAAEGHDLRLERGQEYLILEKNDVHWWRARDKYGNEGYIPSNYVTGKKSNNLDQYEW
YCRNMNRSKAEQLLRSEDKEGGFMVRDSSQPGLYTVSLYTKFGGEGSSGFRHYHIKETTTSPKKYYLAEKHAFGSIPELIEYH
KHNAAGLVTRLRYPVSVKGK
NAPTTAGFSYEKWEINPSELTFMRELGSGLFGVVRLGKWRAQYKVAIKAIREGAMCEEDFIEEAKVMMKLTHPKLVQLYGV
CTQQKPIYIVTEFMERGCLLNFLRQRQGHFSRDVLLSMCQDVCEGMEYLERNSFIHRDLAARNCLVSEAGVVKVSDFGMAR
YVLDDQYTSSSGAKFPVKWCPPEVFNYSRFSSKSDVWSFGVLMWEVFTEGRMPFEKYTNYEVVTMVTRGHRLYQPKLASN
YVYEVMLRCWQEKPEGRPSFEDLLRTIDELVECEETFGR

Cys=449

Figure 21

SEQ ID 3: FULL LENGTH ITK PROTEIN NP_005537 620aa

MNNFILLEEQLIKKSQQKRRTSPSNFKVRFFVLTKASLAYFEDRHGKKRTLKGSIELSRIKCVEIVKSDISIPCHYKYPFQVVHD
NYLLYVFAPDRESRQRWVLALKEETRNNNSLVPKYHPNFWMDGKWRCCSQLEKLATGCAQYDPTKNASKKPLPPTPEDNR
RPLWEPEETVVIALYDYQTNDPQELALRRNEEYCLLDSSEIHWWRVQDRNGHEGYVPSSYLVEKSPNNLETYEWYNKSISR
DKAEKLLLDTGKEGAFMVRDSRTAGTYTVSVFTKAVVSENNPCIKHYHIKETNDNPKRYYVAEKYVFDSIPLLINYHQHNG
GGLVTRLRYPVCFGRQKAPVTAGLRYGKWVIDPSELTFVQEIGSGQFGLVHLGYWLNKDKVAIKTIREGAMSEEDFIEEAEV
MMKLSHPKLVQLYGVCLEQAPICLVFEFMEHGCLSDYLRTQRGLFAAETLLGMCLDVCEGMAYLEEACVIHRDLAARNCL
VGENQVIKVSDFGMTRFVLDDQYTSSTGTKFPVKWASPEVFSFSRYSSKSDVWSFGVLMWEVFSEGKIPYENRSNSEVVEDI
STGFRLYKPRLASTHVYQIMNHCWKERPEDRPAFSRLLRQLAEIAESGL

Cys=442

Figure 22

SEQ ID 4: FULL LENGTH BMX PROTEIN NP_001712 675aa

MDTKSILEELLLKRSQQKKKMSPNNYKERLFVLTKTNLSYYEYDKMKRGSRKGSIEIKKIRCVEKVNLEEQTPVERQYPFQIV
YKDGLLYVYASNEESRSQWLKALQKEIRGNPHLLVKYHSGFFVDGKFLCCQQSCKAAPGCTLWEAYANLHTAVNEEKHRV
PTFPDRVLKIPRAVPVLKMDAPSSSTTLAQYDNESKKNYGSQPPSSSTSLAQYDSNSKKIYGSQPNFNMQYIPREDFPDWWQ
VRKLKSSSSSEDVASSNQKERNVNHTTSKISWEFPESSSSEEEENLDDYDWFAGNISRSQSEQLLRQKGKEGAFMVRNSSQV
GMYTVSLFSKAVNDKKGTVKHYH
VHTNAENKLYLAENYCFDSIPKLIHYHQHNSAGMITRLRHPVSTKANKVPDSVSLGNGIWELKREEITLLKELGSGQFGVVQ
LGKWKGQYDVAVKMIKEGSMSEDEFFQEAQTMMKLSHPKLVKFYGVCSKEYPIYIVTEYISNGCLLNYLRSHGKGLEPSQL
LEMCYDVCEGMAFLESHQFIHRDLAARNCLVDRDLCVKVSDFGMTRYVLDDQYVSSVGTKFPVKWSAPEVFHYFKYSSKS
DVWAFGILMWEVFSLGKQPYDLYDNSQVVLKVSQGHRLYRPHLASDTIYQIMYSCWHELPEKRPTFQQLLSSIEPLREKDK
H

Cys=496

Figure 23

SEQ ID 5: FULL LENGTH TXK PROTEIN NP_003319 527 aa

MILSSYNTIQSVFCCCCCCSVQKRQMRTQISLSTDEELPEKYTQRRRPWLSQLSNKKQSNTGRVQPSKRKPLPPLPPSEVAEE
KIQVKALYDFLPREPCNLALRRAEEYLILEKYNPHWWKARDRLGNEGLIPSNYVTENKITNLEIYEWYHRNITRNQAEHLLR
QESKEGAFIVRDSRHLGSYTISVFMGARRSTEAAIKHYQIKKNDSGQWYVAERHAFQSIPELIWYHQHNAAGLMTRLRYPVG
LMGSCLPATAGFSYEKWEIDPSELAFIKEIGSGQFGVVHLGEWRSHIQVAIKAINEGSMSEEDFIEEAKVMMKLSHSKLVQLY
GVCIQRKPLYIVTEFMENGCLLNYLRENKGKLRKEMLLSVCQDICEGMEYLERNGYIHRDLAARNCLVSSTCIVKISDFGMT
RYVLDDEYVSSFGAKFPIKWSPPEVFLFNKYSSKSDVWSFGVLMWEVFTEGKMPFENKSNLQVVEAISEGFRLYRPHLAPMS
IYEVMYSCWHEKPEGRPTFAELLRAVTEIAETW

Cys 350

Figure 24

SEQ ID 6: FULL LENGTH JAK3 PROTEIN NP_000206 1124 aa

MAPPSEETPLIPQRSCSLLSTEAGALHVLLPARGPGPPQRLSFSFGDHLAEDLCVQAAKASGILPVYHSLFALATEDLSCWFPP
SHIFSVEDASTQVLLYRIRFYFPNWFGLEKCHRFGLRKDLASAILDLPVLEHLFAQHRSDLVSGRLPVGLSLKEQGECLSLAV
LDLARMAREQAQRPGELLKTVSYKACLPPSLRDLIQGLSFVTRRRIRRTVRRALRRVAACQADRHSLMAKYIMDLERLDPA
GAAETFHVGLPGALGGHDGLGLLRVAGDGGIAWTQGEQEVLQPFCDFPEIVDISIKQAPRVGPAGEHRLVTVTRTDNQILEA
EFPGLPEALSFVALVDGYFRLTTDSQHFFCKEVAPPRLLEEVAEQCHGPITLDFAINKLKTGGSRPGSYVLRRSPQDFDSFLLT
VCVQNPLGPDYKGCLIRRSPTGTFLLVGLSRPHSSLRELLATCWDGGLHVDGVAVTLTSCCIPRPKEKSNLIVVQRGHSPPTS
SLVQPQSQYQLSQMTFHKIPADSLEWHENLGHGSFTKIYRGCRHEVVDGEARKTEVLLKVMDAKHKNCMESFLEAASLMS
QVSYRHLVLLHGVCMAGDSTMVQEFVHLGAIDMYLRKRGHLVPASWKLQVVKQLAYALNYLEDKGLPHGNVSARKVLL
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YEDRQQLPAPKWTELALLIQQCMAYEPVQRPSFRAVIRDLNSLISSDYELLSDPTPGALAPRDGLWNGAQLYACQDPTIFEER
HLKYISQLGKGNFGSVELCRYDPLGDNTGALVAVKQLQHSGPDQQRDFQREIQILKALHSDFIVKYRGVSYGPGRQSLRLV
MEYLPSGCLRDFLQRHRARLDASRLLLYSSQICKGMEYLGSRRCVHRDLAARNILVESEAHVKIADFGLAKLLPLDKDYYV
VREPGQSPIFWYAPESLSDNIFSRQSDVWSFGVVLYELFTYCDKSCSPSAEFLRMMGCERDVPALCRLLELLEEGQRLPAPPA
CPAEVHELMKLCWAPSPQDRPSFSALGPQLDMLWSGSRGCETHAFTAHPEGKHHSLSFS

Cys=909

Figure 25