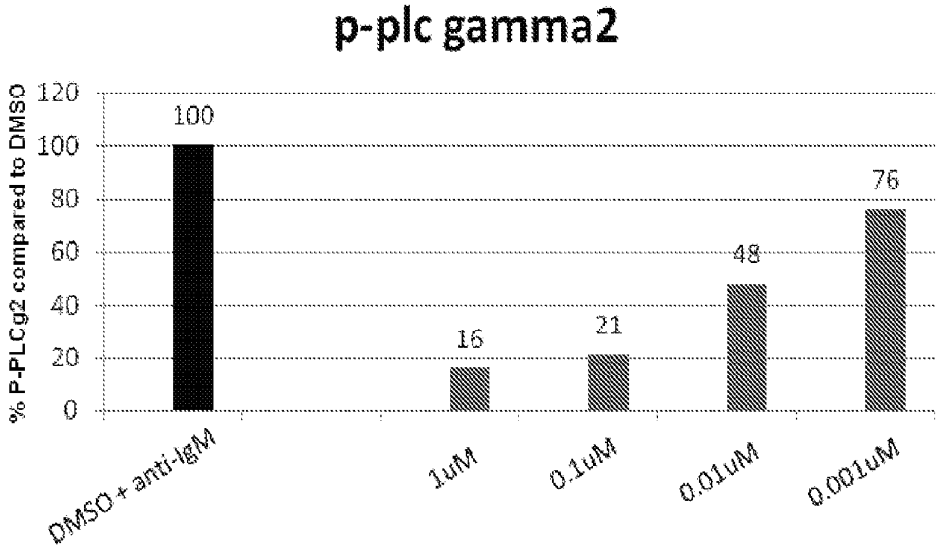
Dose-Response with Compound **I-2** in Ramos cells



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

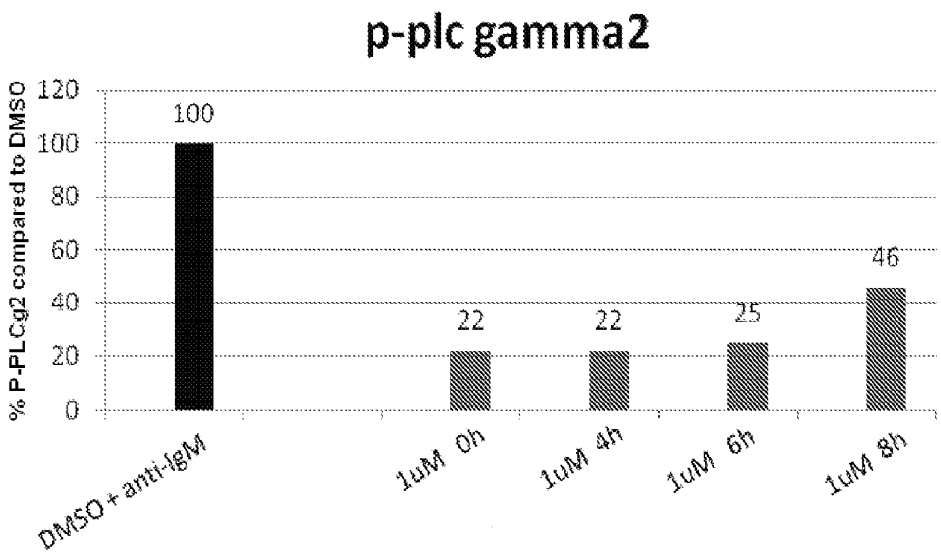
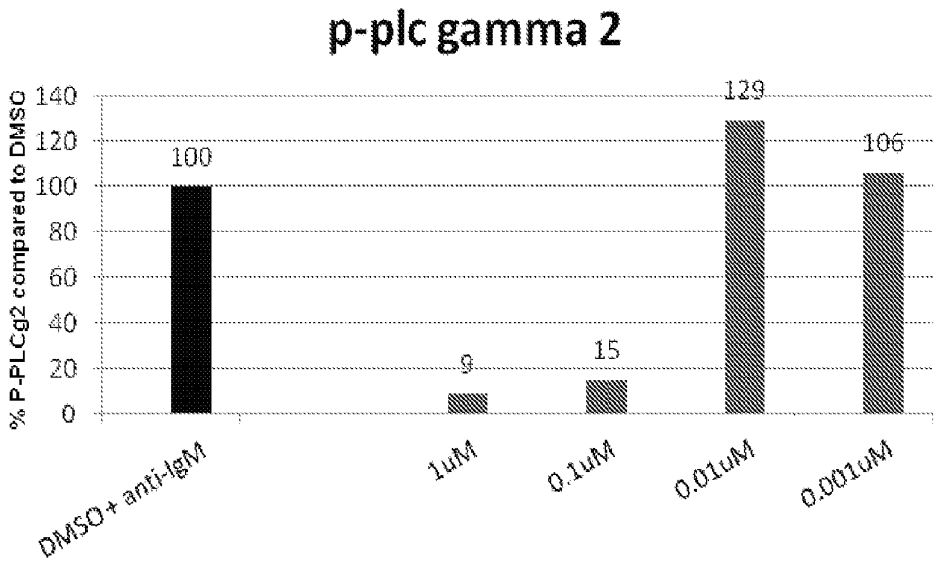


Figure 1

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



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

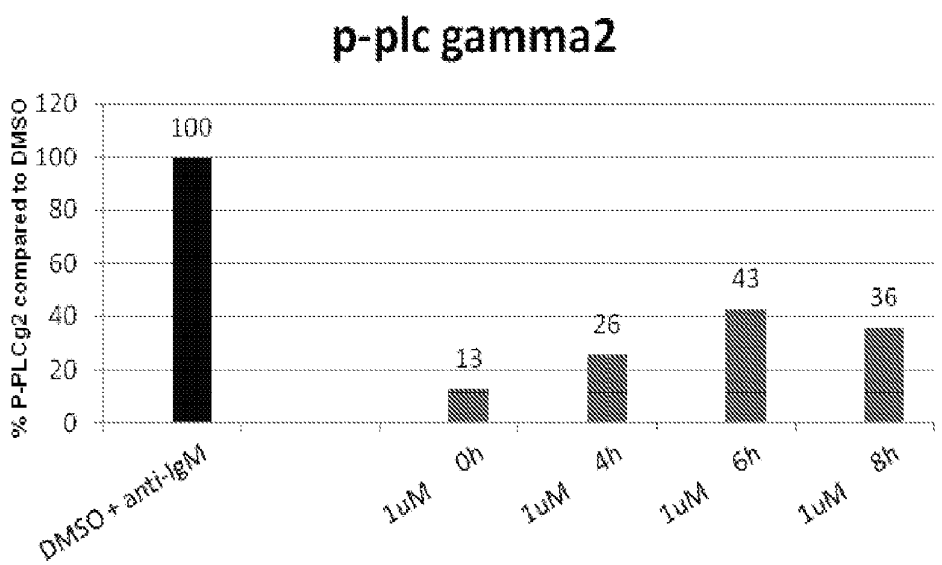
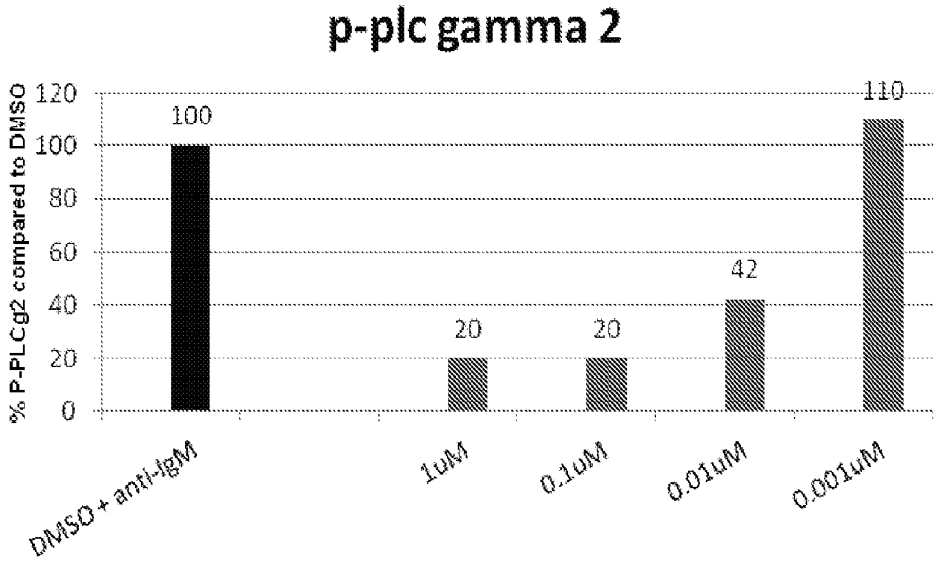


Figure 2

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



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

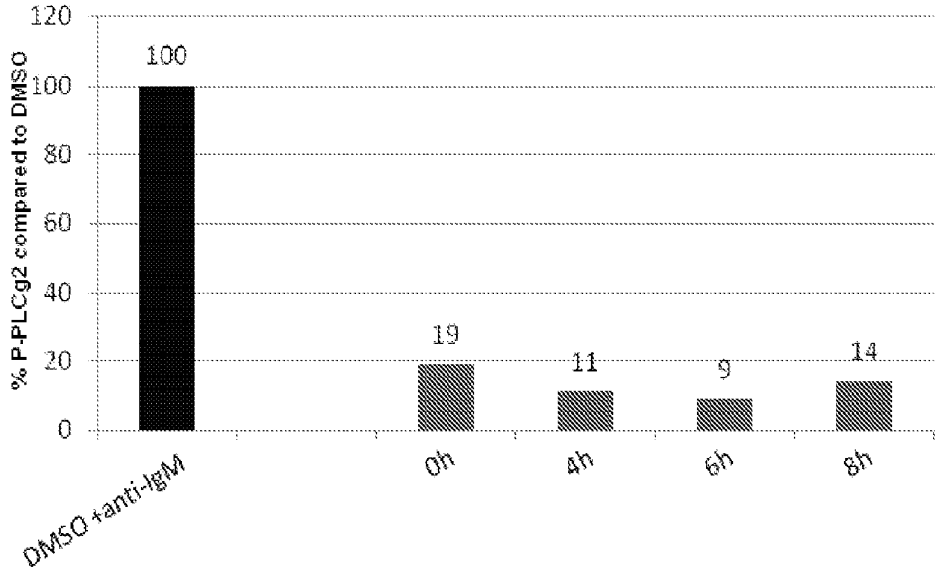


Figure 3

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

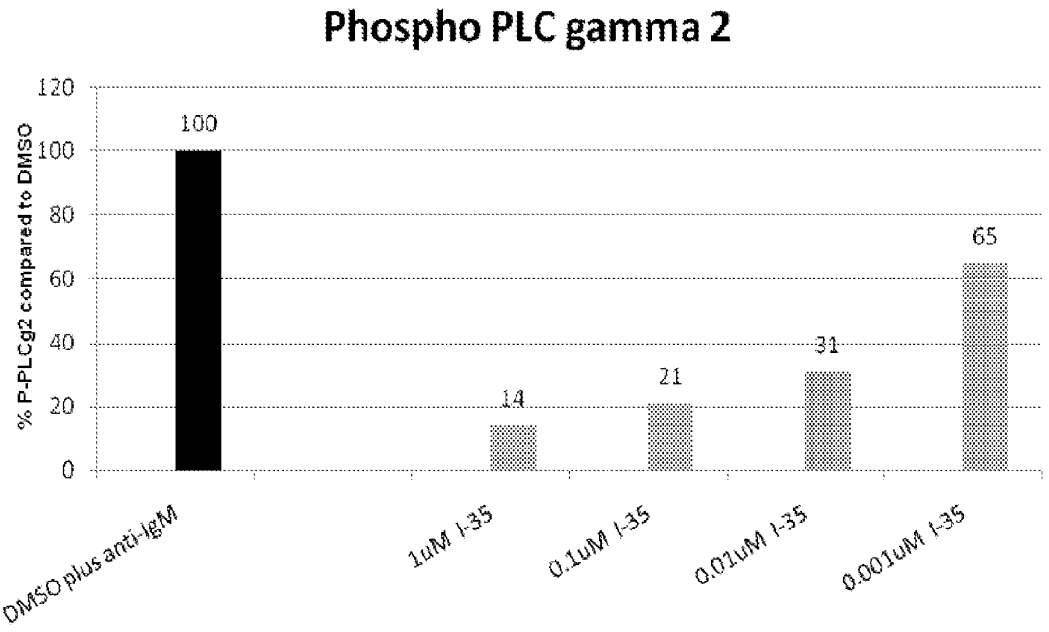


Figure 4

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

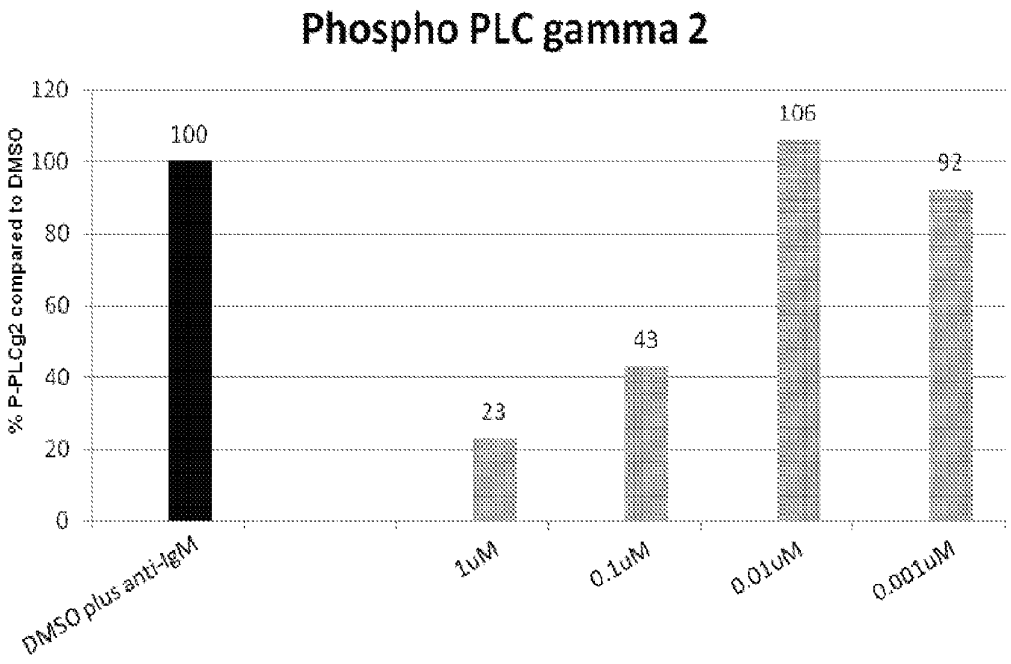


Figure 5

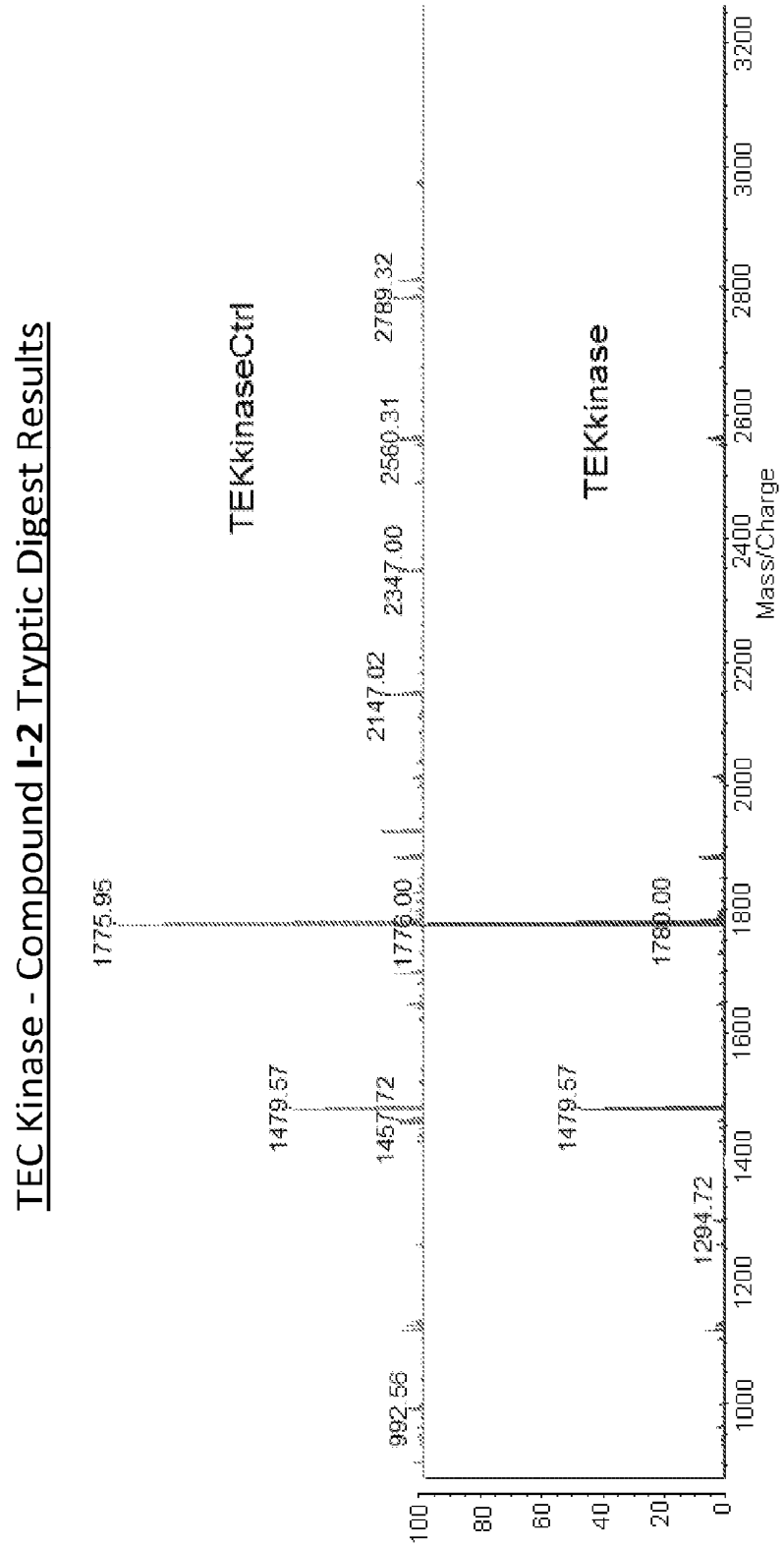

TEC Kinase - Compound I-2 Tryptic Digest Results
TEKkinaseCtrl
TEKkinase
1775.95
1776.00
1780.00
2147.02
2347.00
2560.31
2789.32
1479.57
1457.72
1479.57
1294.72
992.56
Mass/Charge
100
80
60
40
20
0
1000
1200
1400
1600
1800
2000
2200
2400
2600
2800
3000
3200

Figure 6

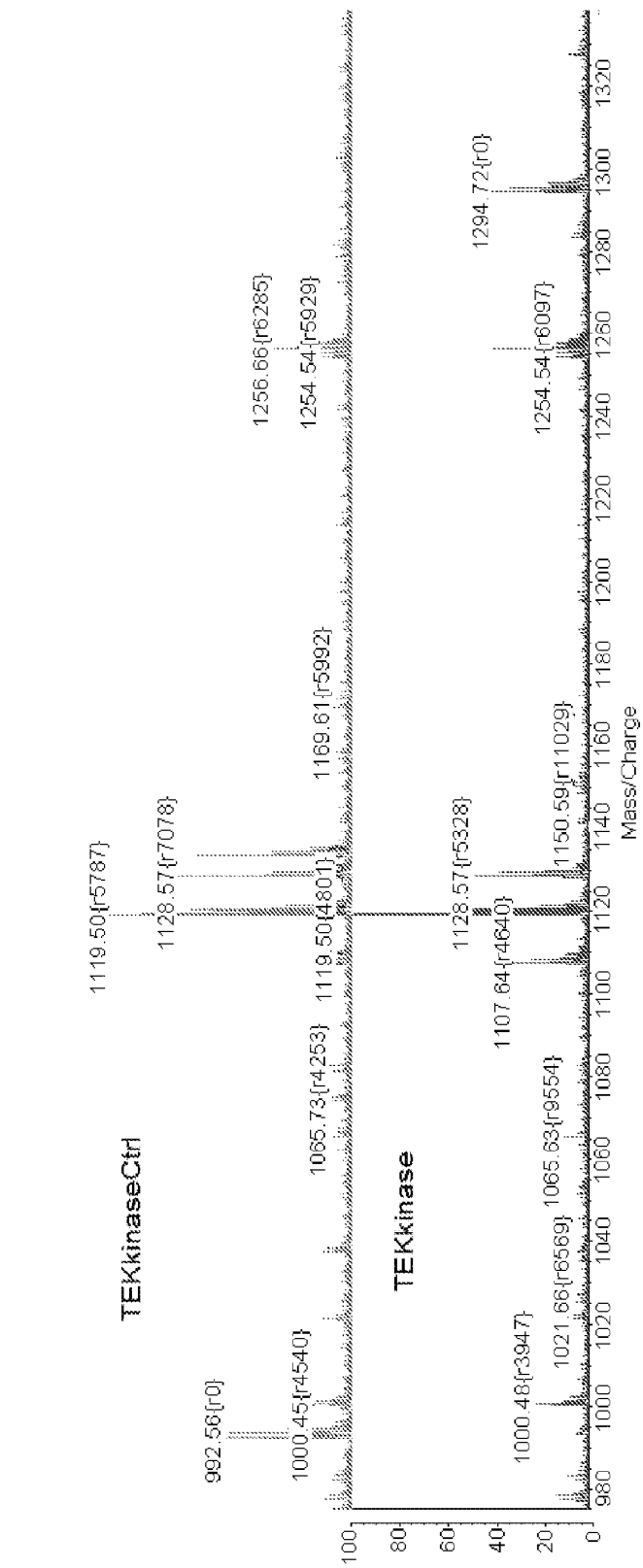


Figure 6 (continued)

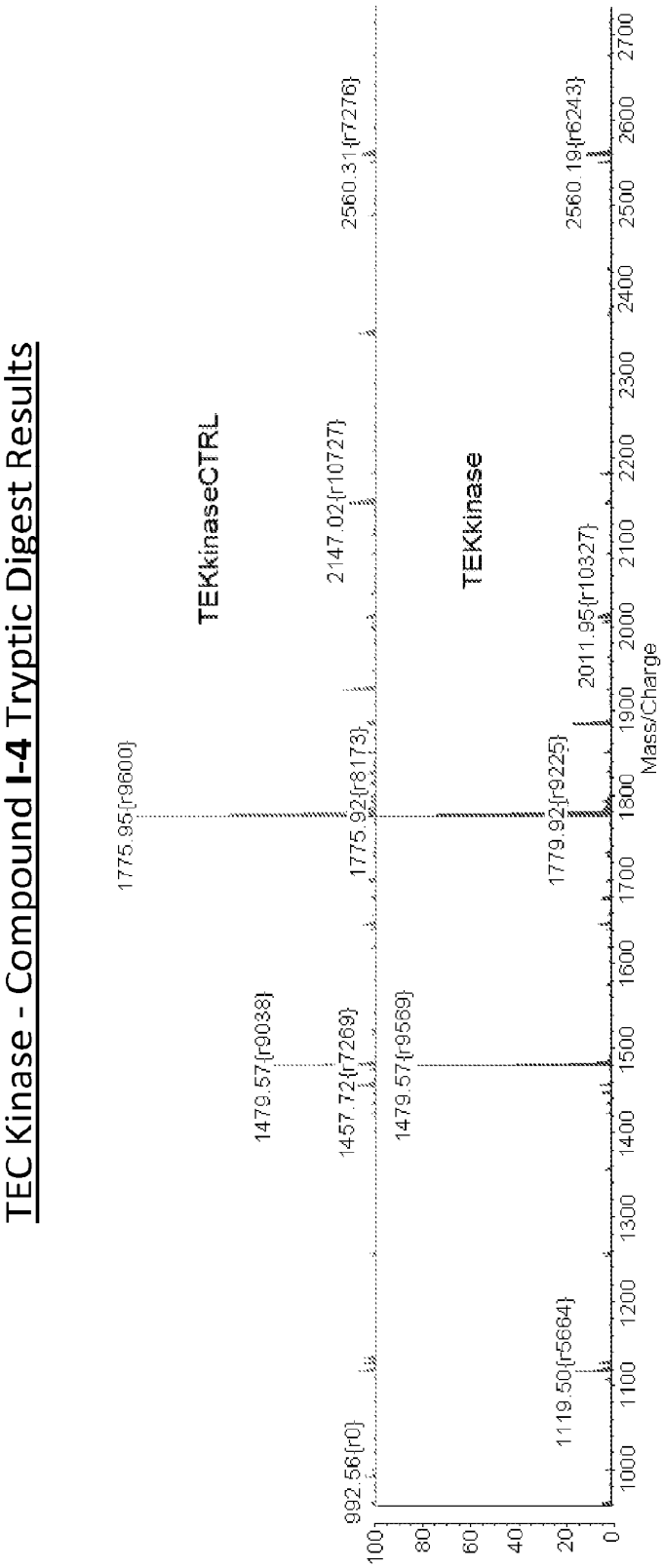

TEC Kinase - Compound I-4 Tryptic Digest Results
TEKkinaseCTRL
TEKkinase
992.56{r0}
1119.50{r5664}
1457.72{r7269}
1479.57{r9038}
1479.57{r9569}
1775.95{r9600}
1775.92{r8173}
1779.92{r9225}
2011.95{r10327}
2147.02{r10727}
2560.31{r7276}
2560.19{r6243}
Mass/Charge

Figure 7

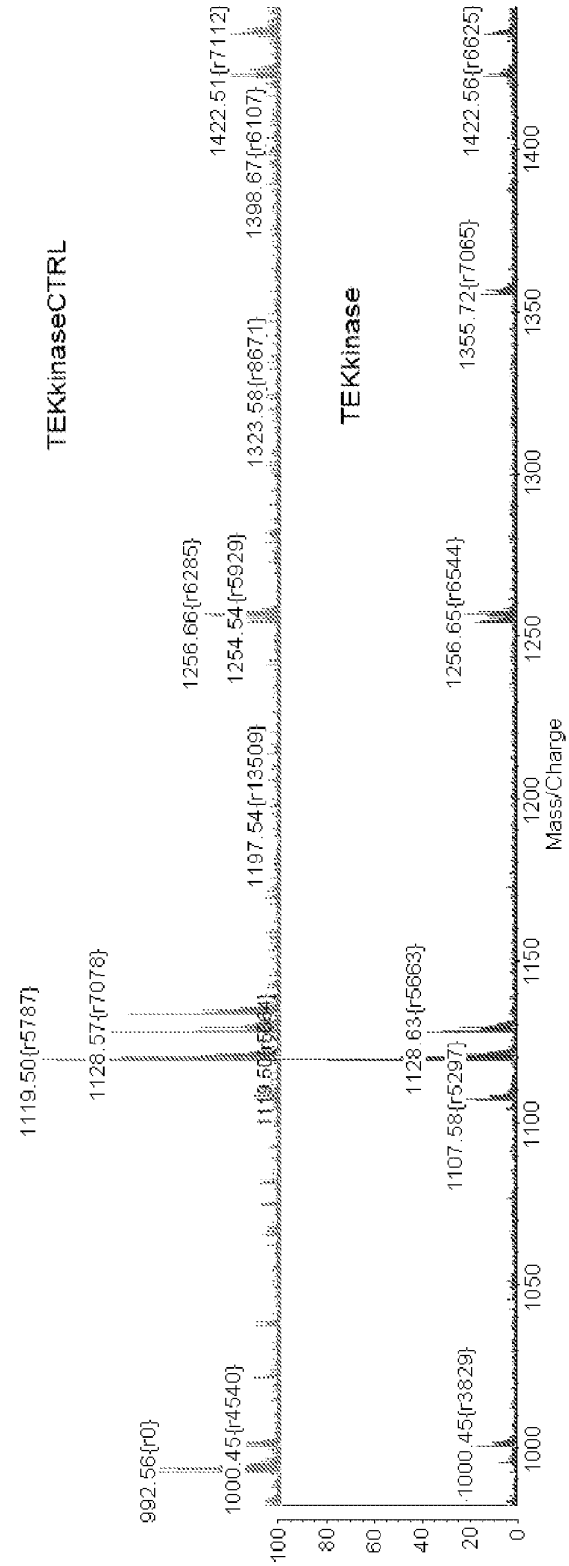


Figure 7 (continued)

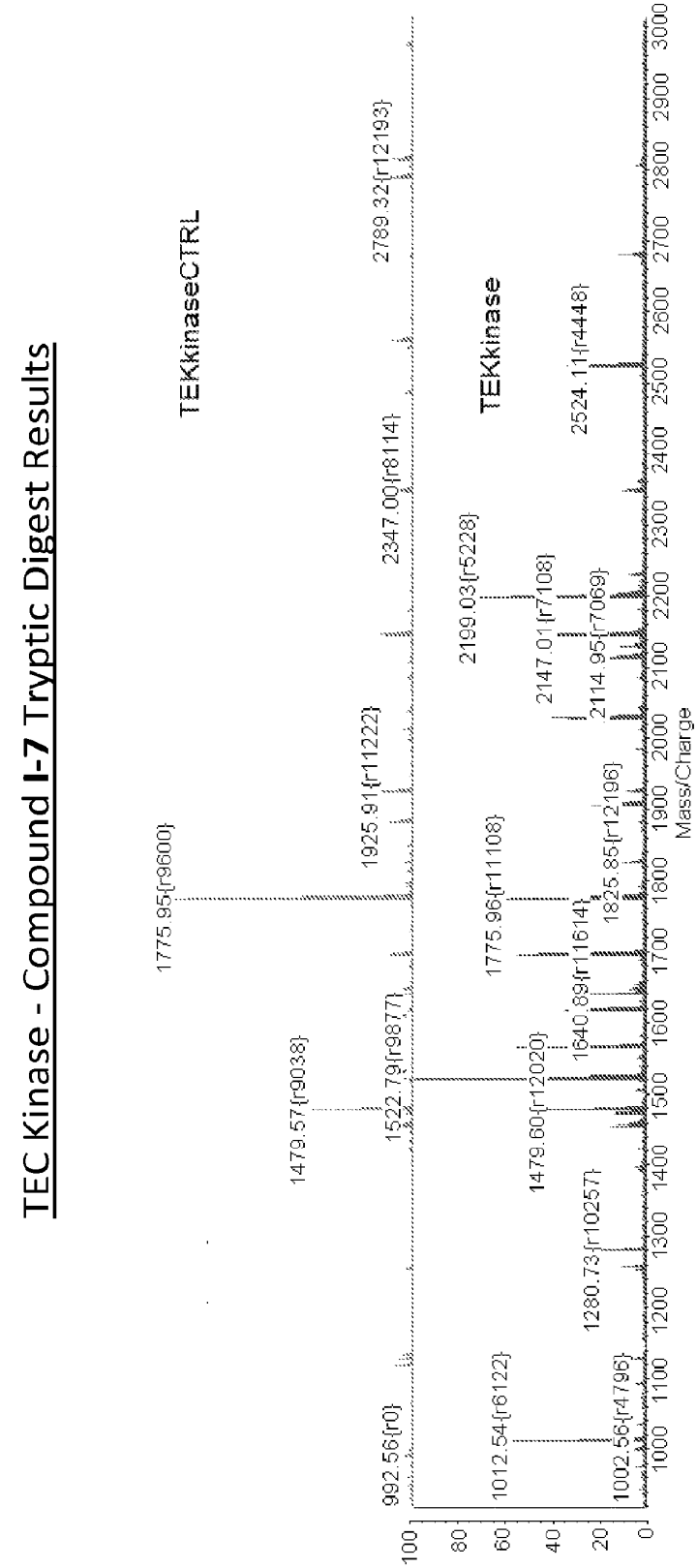

TEC Kinase - Compound I-7 Tryptic Digest Results
TEKkinaseCTRL
TEKkinase
992.56{r0}
1012.54{r6122}
1002.56{r4796}
1479.57{r9038}
1280.73{r10257}
1522.79{r9877}
1479.60{r12020}
1640.89{r11614}
1775.95{r9600}
1775.96{r11108}
1825.85{r12196}
1925.91{r11222}
2114.95{r7069}
2147.01{r7108}
2199.03{r5228}
2347.00{r8114}
2524.11{r4448}
2789.32{r12193}
Mass/Charge

Figure 8

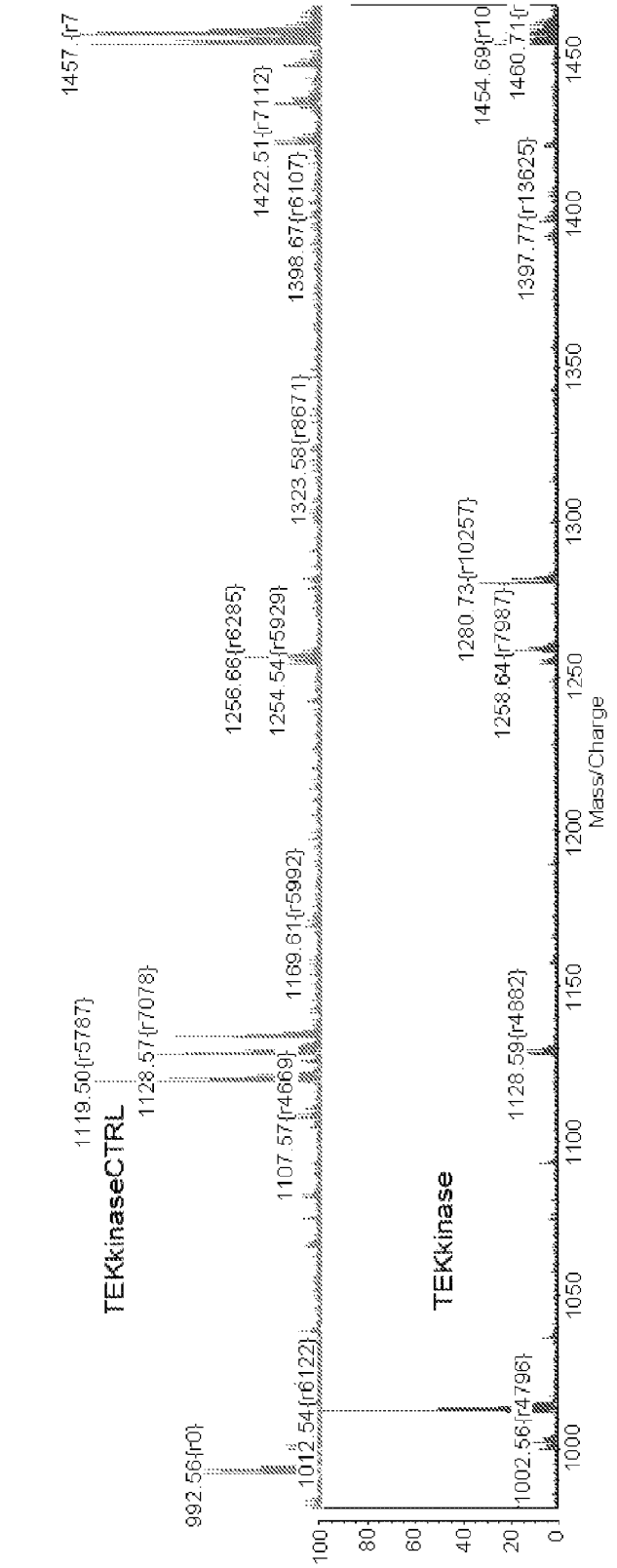


Figure 8 (continued)

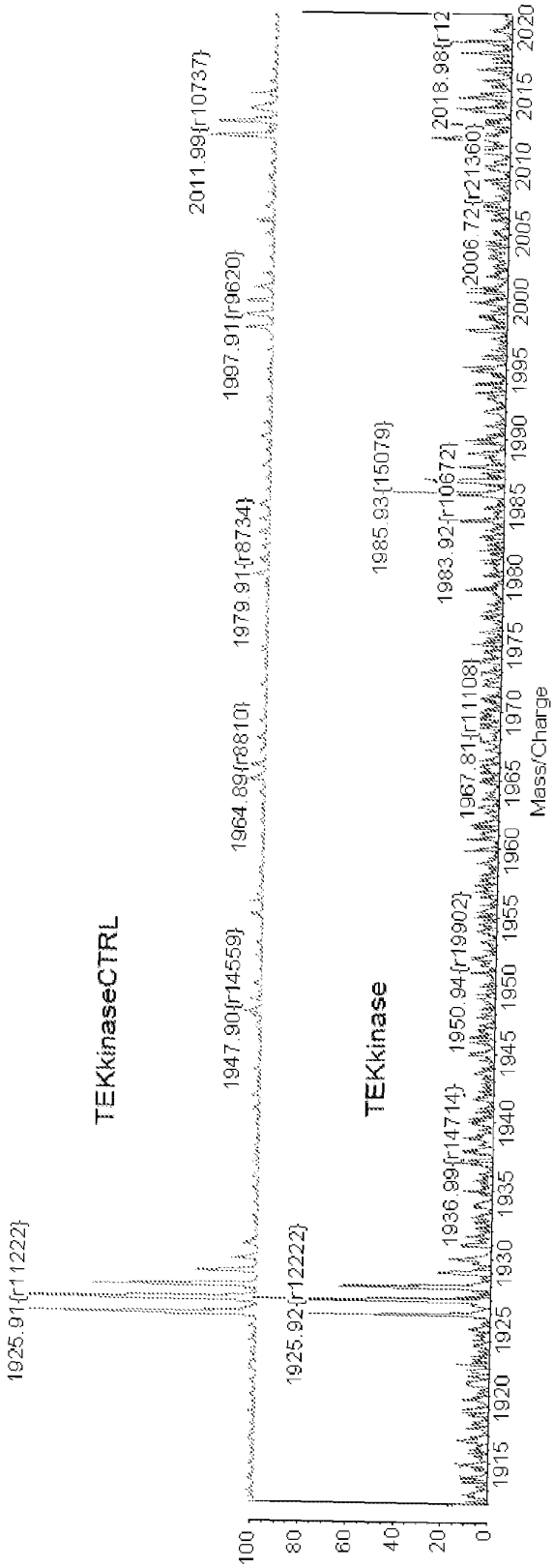
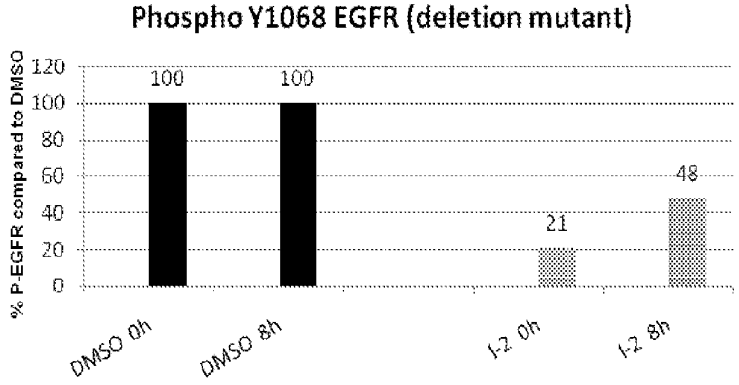
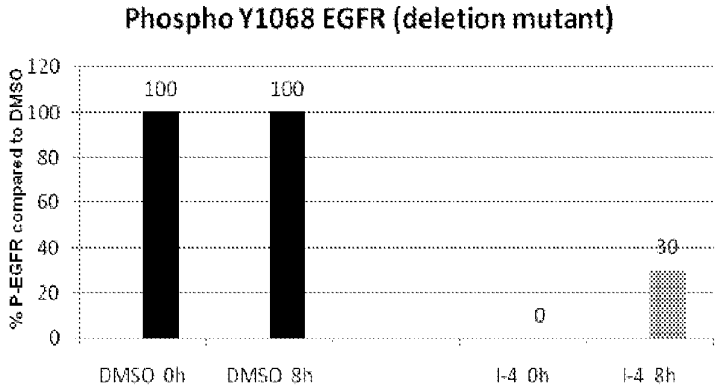


Figure 8 (continued)

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





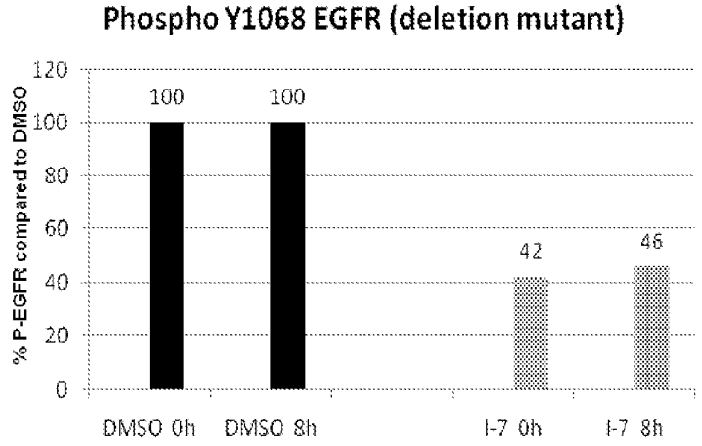


Figure 9

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

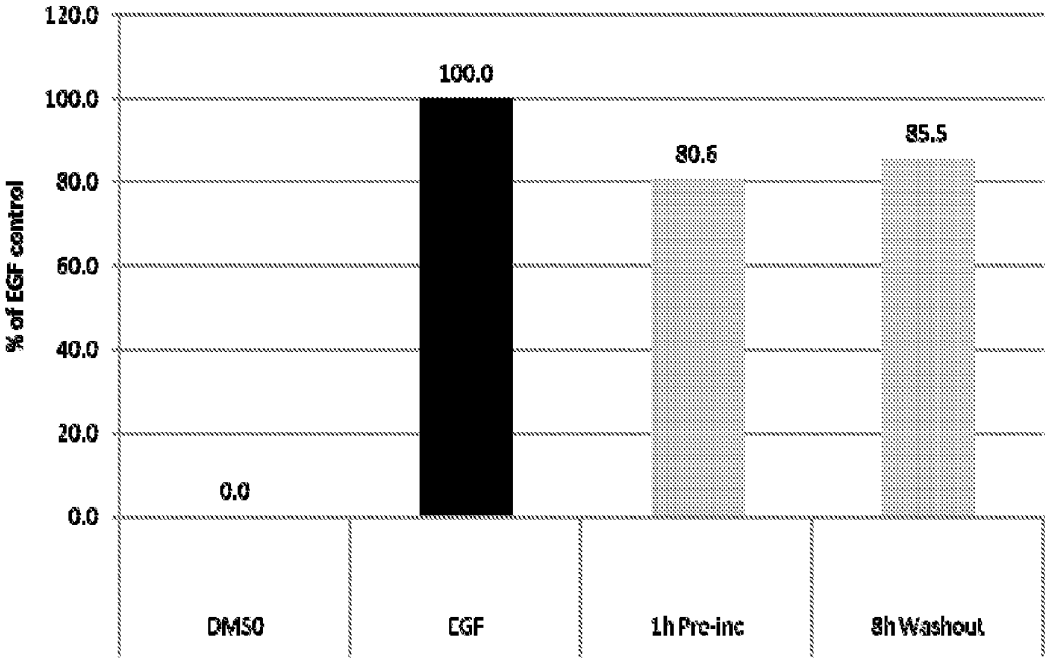


Figure 10

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

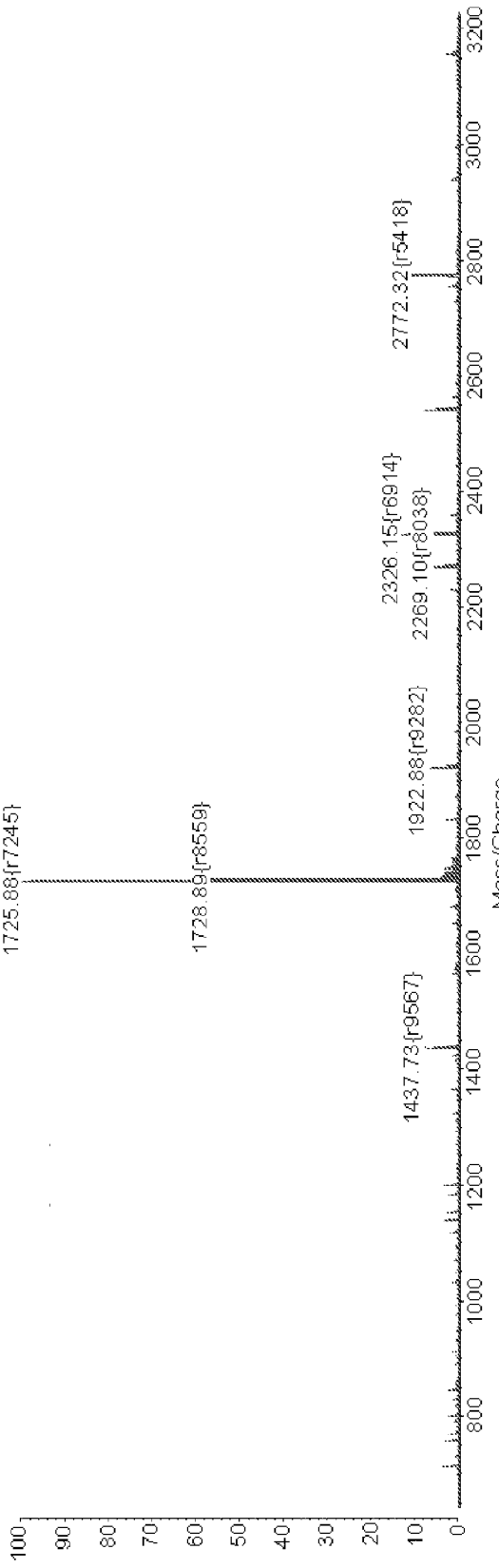


Figure 11

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

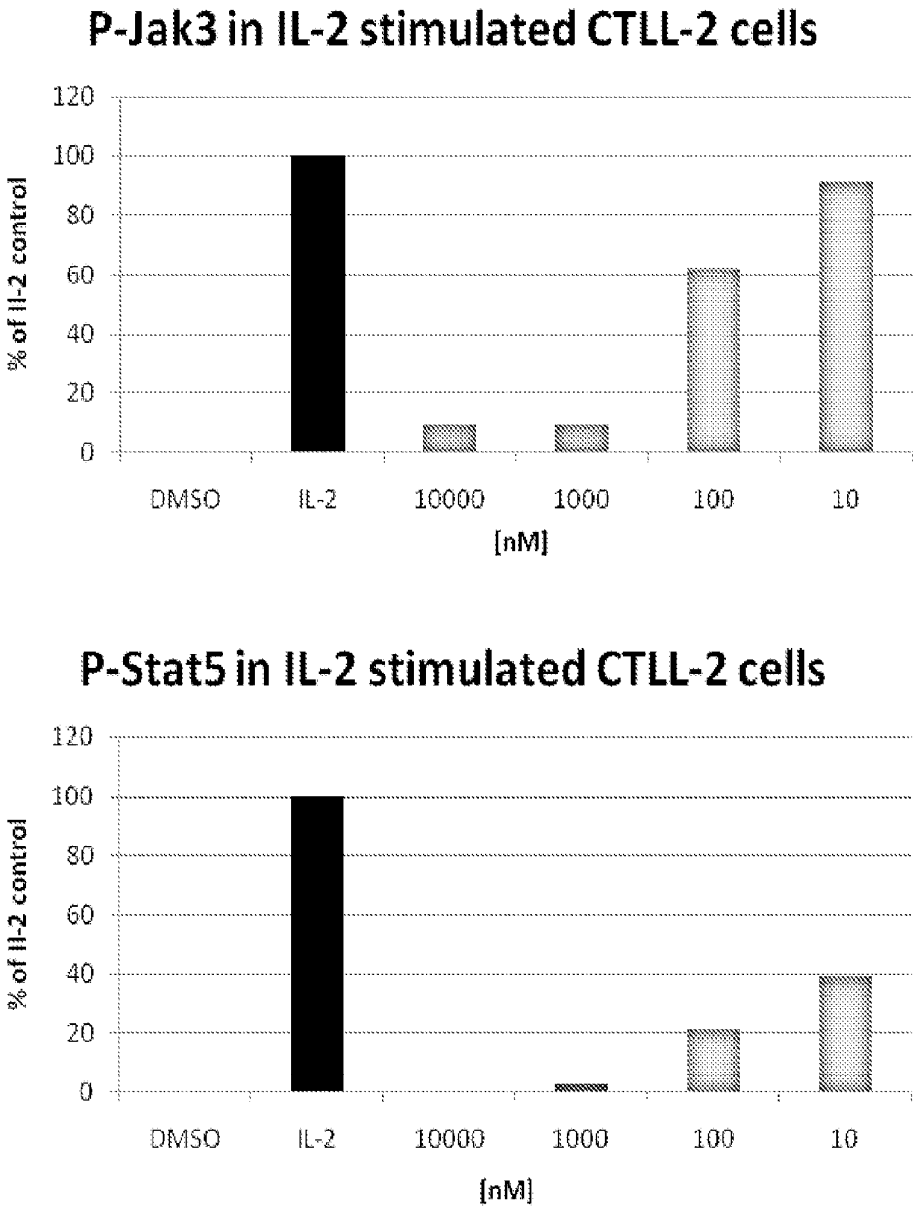
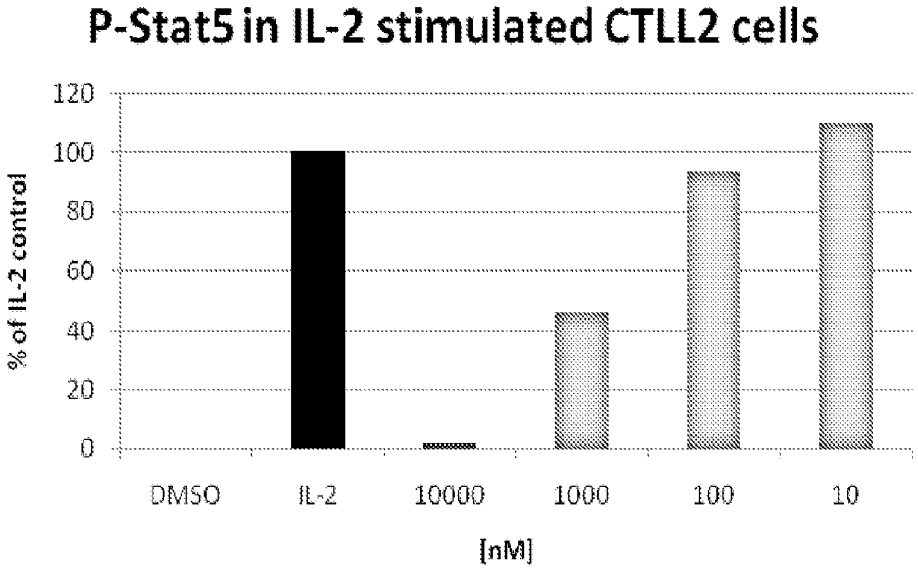


Figure 12

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



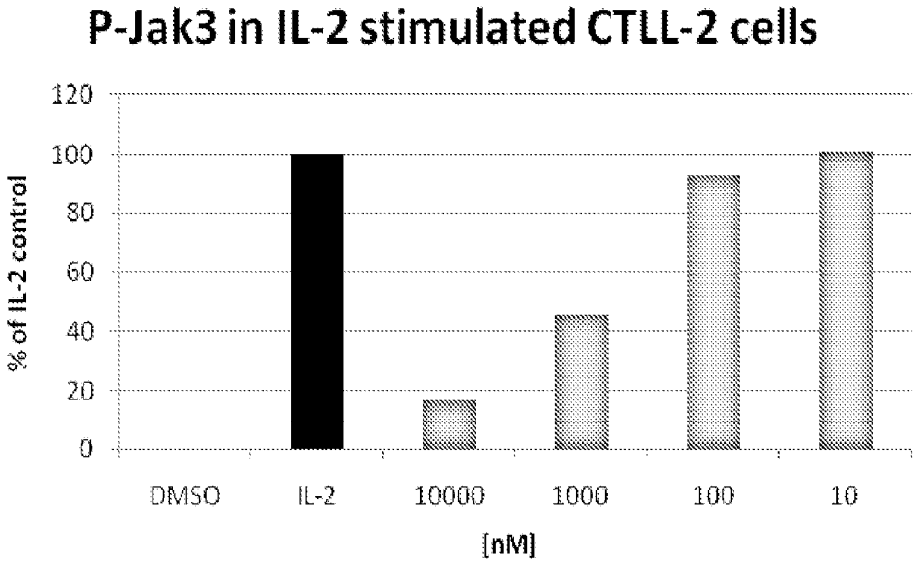


Figure 13

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

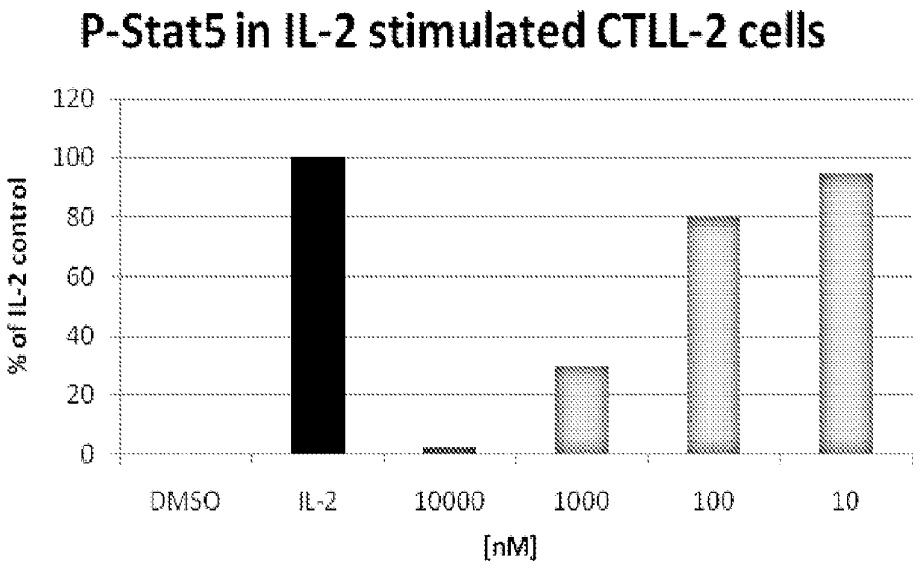


Figure 14

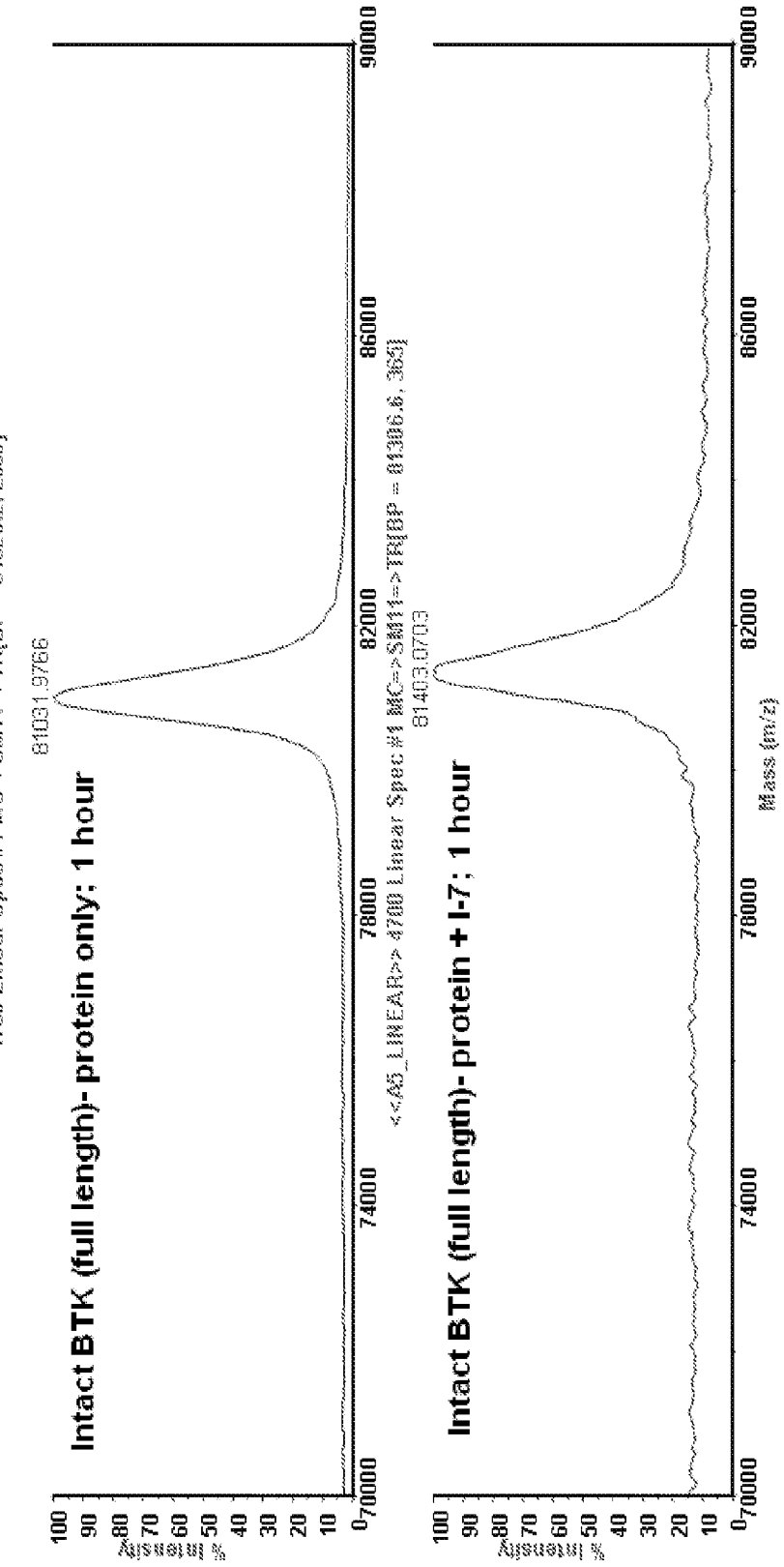

Intact BTK (full length)- protein only; 1 hour
Intact BTK (full length)- protein + I-7; 1 hour
% Intensity
Mass (m/z)
70000
74000
78000
82000
86000
90000
0
10
20
30
40
50
60
70
80
90
100

Figure 15

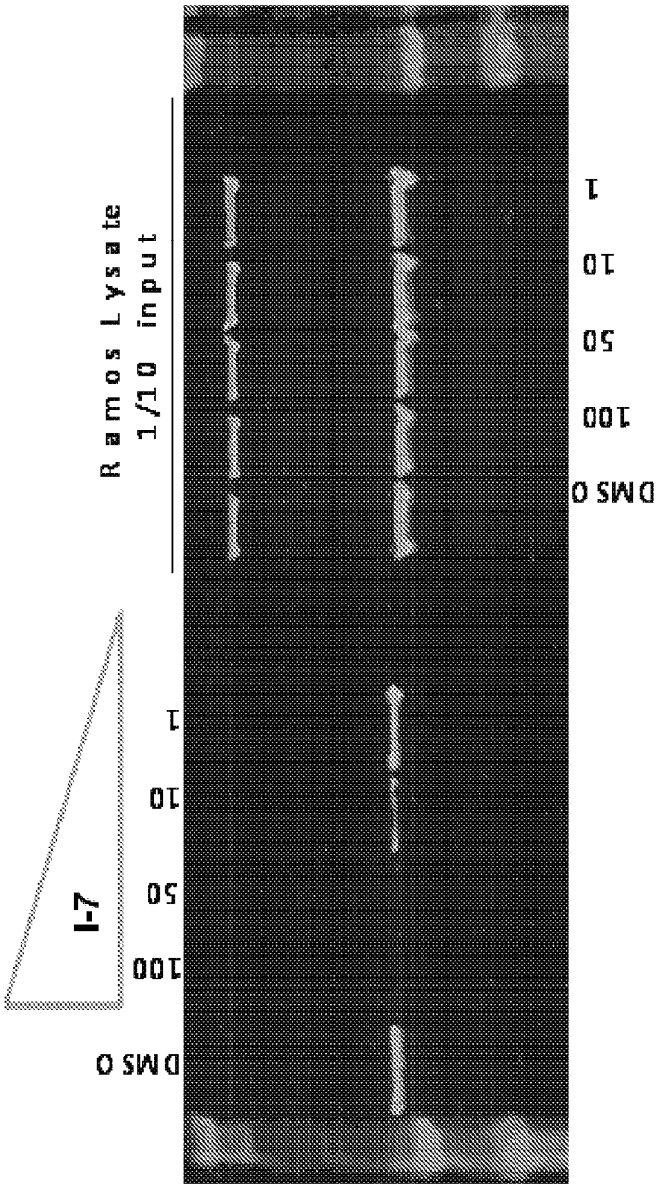


Figure 16

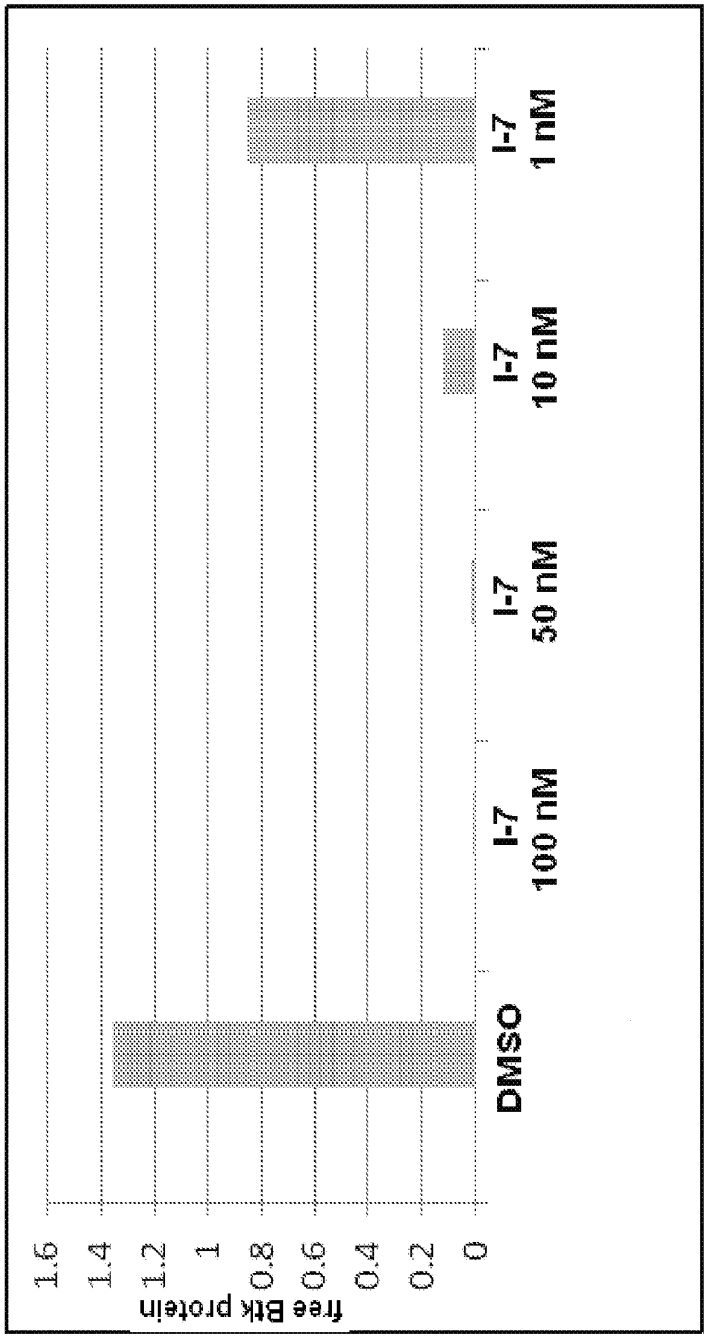

free Btk protein
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
DMSO
I-7 100 nM
I-7 50 nM
I-7 10 nM
I-7 1 nM

Figure 17

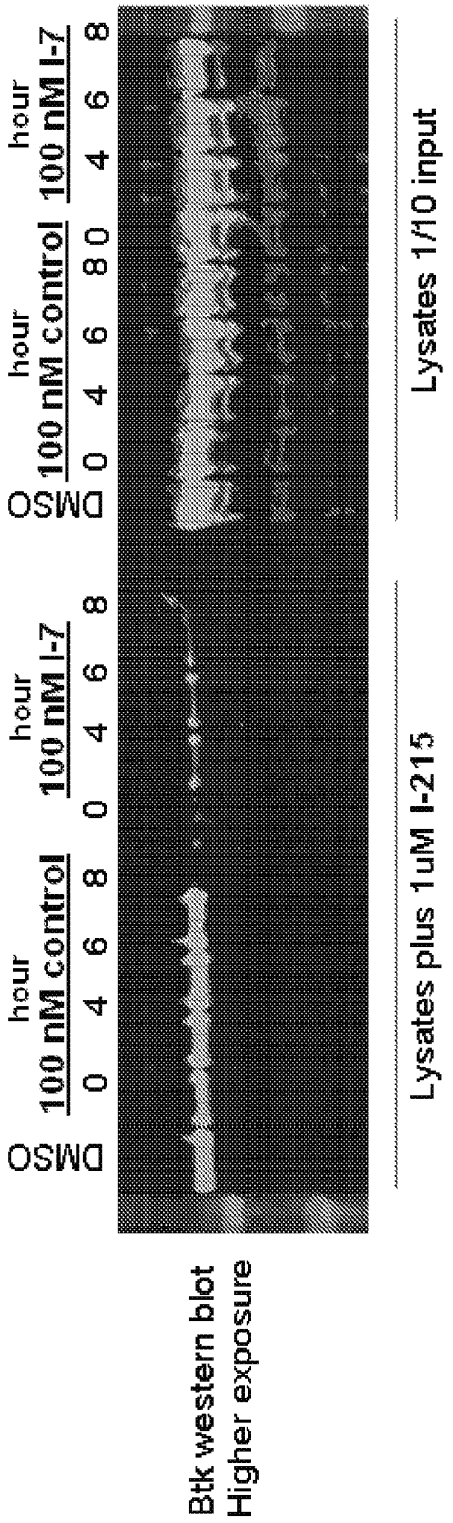

DMSO
hour
100 nM control
0 4 6 8
hour
100 nM I-7
0 4 6 8
DMSO
hour
100 nM control
0 4 6 8
hour
100 nM I-7
0 4 6 8
Lysates plus 1uM I-215
Lysates 1/10 input
Btk western blot
Higher exposure

Figure 18

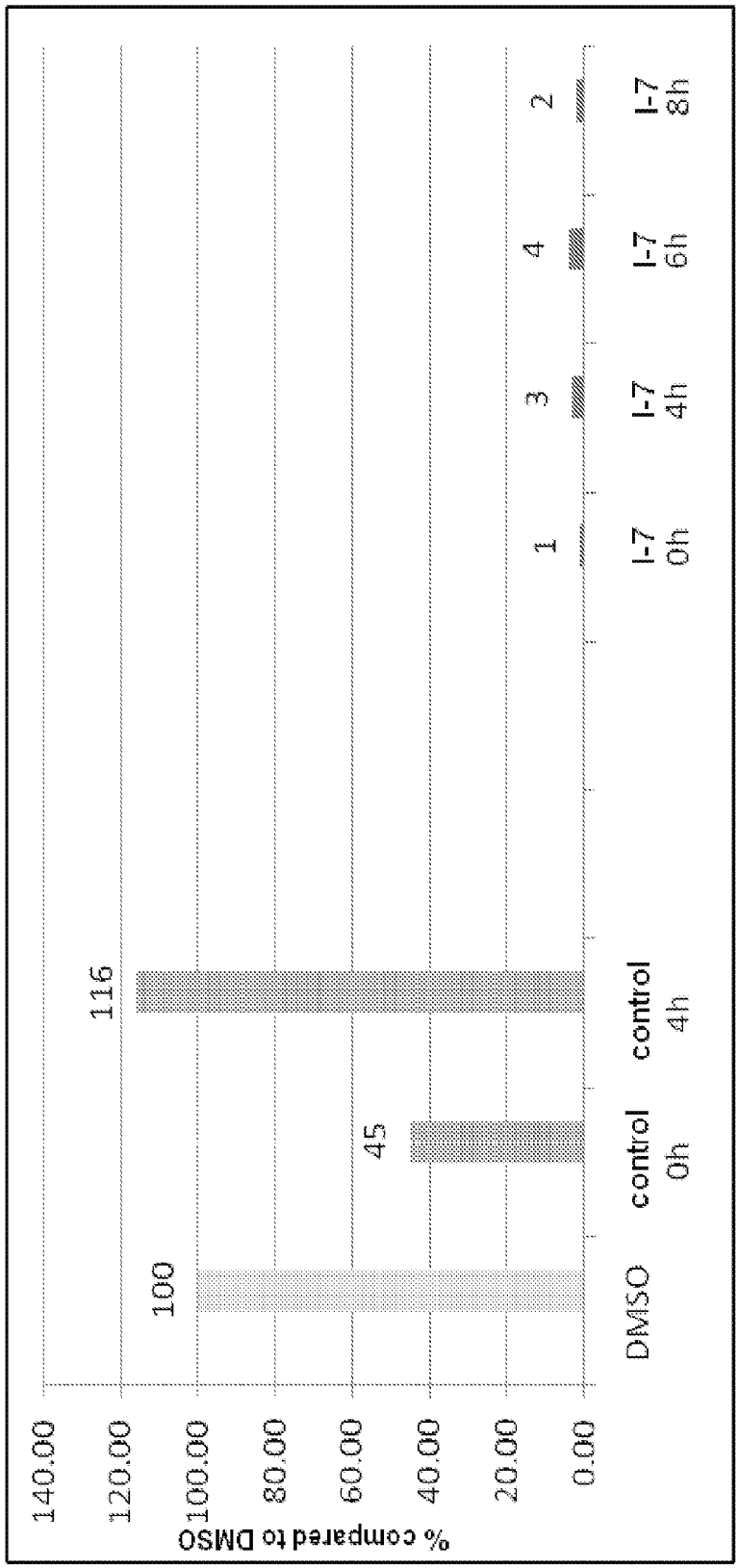

% compared to DMSO
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
100
45
116
DMSO
control 0h
control 4h
1
I-7 0h
3
I-7 4h
4
I-7 6h
2
I-7 8h

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
YCRNMNRSKAEQLLRSEDKEGGFMVRDSSQPGLYTVSLYTKFGGEGSSGFRHYHIKETTTSPKKYYLAEKHAFGSIPEIIEYH
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

MILSSYNTIQSVFCCCCCSVQKRQMRTQISLSTDEELPEKYTQRRRPWLSQLSNKKQSNTGRVQPSKRKPLPPLPPSEVAEE
KIQVKALYDFLPREPCNLALRRAEEYLILEKYNPHWWKARDRLGNEGLIPSNYVVTENKITNLEIYEWYHRNITRNQAEHLLR
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
AREGADGSPPFIKLSDPGVSPAVLSLEMLTDRIPWVAPECLREAQTLSLEADKWGFGATVWEVFSGVTMPISALDPAKKLQF
YEDRQQLPAPKWTELALLIQQCMAYEPVQRPSFRAVIRDLNSLISSDYELLSDPTPGALAPRDGLWNGAQLYACQDPTIFEER
HLKYISQLGKGNFGSVELCRYDPLGDNTGALVAVKQLQHSGPDQQRDFQREIQILKALHSDFIVKYRGVSYGPGRQSLRLV
MEYLPSGCLRDFLQRHRARLDASRLLLYSSQICKGMEYLGSRRCVHRDLAARNILVESEAHVKIADFGLAKLLPLDKDYYV
VREPGQSPIFWYAPESLSDNIFSRQSDVWSFGVVLYELFTYCDKSCSPSAEFLRMMGCERDVPALCRLLELLEEGQRLPAPPA
CPAEVHELMKLCWAPSPQDRPSFSALGPQLDMLWSGSRGCETHAFTAHPEGKHHSLSFS

Cys=909

Figure 25