

rabbit serum. (d) Serum starved-HeLa cells were treated with PMA or TNF α and lysed at the indicated times. IKK and NAK activities were determined by immunocomplex kinase assays using GST-I κ B α or GST-IKK β as substrates. (e) Serum starved-A172 cells were treated with 100 ng/ml PDGF in the presence or absence of Ro 31-8220 (5 μ M), and lysed at the indicated times. NAK activity was determined by immunocomplex kinase assay. (f) Increasing amounts of NAK(KM)(2.0, 3.0, and 4.0 μ g/plate) were transfected with NF- κ B-Luc and actin- β gal reporters into A172 cells and, 30 hours after transfection, cells were treated with PDGF for additional 6h. Luciferase activity was determined as described above.

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NAK      1  ---- -OSTANHLWLLSILGCGTANVGGHKKRCMFFAKVFNKISFLEPVVQMF
IKK-1    1  ---- -OSTANVWHTDIDLGGCTASVSSARMKKSLWAKRINTASYVREVOVR
IKKα     1  MERPPGRFGAGGPWEMKRLGGGFONVCIYOHKEIKIAINSORLEBLSTKNERWCH
IKKβ     1  MSWSPSSTQTCTGAWEMKRLGGGFONVIRWNNQETGEGQIAIKQCRQELSPRNERWCL

NAK      55  DFEVLRKLNHKNRVLKFAEEET----TRHKVLIEMKPCGSLYTVLEFSNAYGLPES
IKK-1    55  DFEVLRKLNHQHNVKLFKAVEETG----GRQKVLVMEYCSSSLLSVLESPEAAGLPED
IKKα     61  FIOIKKLNHANVACLVPFENLINDYFLLAMFYCGGDEIRKLNKPFNCGGKRFK
IKKβ     61  FIQIMERTTTPNVAARDLVPFEGMXXNRPANDEFLLAMFYCGGDDIRRYFNQFENCGGLRFG

NAK      111  DFLIKLRLNVLGGMNHLENGIHRDVKPFGNIRVVGEGQSVYKADFGAARELDDEQY
IKK-1    111  DFLVYLRCYVAGMNHLENGIHRDVKPFGNIMRLVGEQSVYKADFGAARELDDEKFL
IKKα     120  QILSLSDYSGSRYLLENKIHRDVKPFGNIVLQDVGCKIIFRDLGLYAKVCGGSLC
IKKβ     121  AILTLSDYSSARYLLENKIHRDVKPFGNIVLQDVG-QRLIHKDLGLYAKVLEGGSLC

NAK      171  VSLYGTVEYLHPDHYERAVLRKDQKKYGATVDLWSICVTFYHAAVGSLLPFRPEGPRRN
IKK-1    171  VSVYGTVEYLHPDHYERAVLRKQKATGATVDLWSICVTFYHAAVGSLLPFRPEGPRRN
IKKα     179  TSFVGTLOYLAPRLE-----NKKPYTAVDYSWSEGTMYEELIAGYRPFLLHLQPF---
IKKβ     180  TSFVGTLOYLAPRLE-----QKKYTVVDYSWSEGTLAEECIKGFRRPFLENWQPF---

NAK      231  KEVMMIITCKPSGALSQVOKAENGFIDSGDMPVSCFLSRGLQVLLTPVLANXLEAM--
IKK-1    231  KEIIMYITCKPSGALSAQAORRNGPVEVYTLBATCQPSLGLQVLLTPVLANXLEAM--
IKKα     228  -FTWHEIKKKDKKICFACPEMSEVRSSEHLEFQNSLCSLQVLLTPVLANXLEAM--
IKKβ     229  -VQWHSKVQKSEVDIVVSEQLNGTVKSSSLFVFNLSVLAERLEKWLQLMLWHPR

NAK      289  QEK-----CWGFDQFAEWSDLIHRMVVVFSLQMLAHKIYIYSYNTAVIFHLMYKQT
IKK-1    289  QAK-----CWGFDQFAETSDLIHRVVVNFSLQVHHIYIHAHNTKAIKFAEYKQT
IKKα     286  QRGPVVLTLPKOPRCVIMDHLILNLKLVHKLNMESAKTISFLLPDLHSLQSLERET
IKKβ     287  QRG--TDFTXGPGCGSKALDILNLKLVHKLNMVTEHTIYPTDESLQSLKREXQQDI

NAK      344  KTISSNDFLYEGRRLVLEPGKLAQHEP-----HTTEENRSEVYSRE---PLNTLQL
IKK-1    344  SVAPRHDEYLFEGHLCVLEPSVSAQHIA-----HTTASSRVLDFSTA---IPKGVAF
IKKα     346  GINFGSDELLSEFGTSIDPRKEAQCVDG---VKGCCDSEHLEDFSKYVGGFAS
IKKβ     345  GTFEEDDELLQERAGLAIIPDKEATDCHSDGKLINEGHLQDMDLVFDFNSKITVETQISF

NAK      393  IYEKISLSPKYPHPRYDLDGDASMAKATGVVCYACRSTASTILL---YQELMRKLERWLE
IKK-1    393  RDPALDVPKFPVKVDLQADNNTAKGVLGAGQALRRANALLD---GQELMRKLERWLE
IKKα     401  RSLSDCVNYLVDQSKHLPILDLKLVMAEAVHYVSGILREDDYSRLGGORAAMSLHYNNA
IKKβ     104  RFPQPEVSCILQEPKRNLAFFDLKRVWGVHHSQTLKEDCNRLGGORAAMNLLKANS

NAK      449  PKDDYNEFHKKKTEWVITLQDQIRNTEKTKVVEKIKKINLEAEEG-EISEHHTALE
IKK-1    449  VQATCRRLEWATSLYLHSSLGERRFSSVAGYPERQELKAADEHRSRLRSTAGVLSR
IKKα     461  NLRMKNMLISASQQLKRLRFFHKSIGDLEKYSQITAGIASEHKLKAWMEESAIH
IKKβ     464  CLRMKNMAMASQQLKAKLDPEKTSIQDLEKYSQTEAGISDQKLLLAWREMEQAVEL

NAK      508  LSS--SQTLETSLODQDRRSPGGSLADAWAHQEGTTPKDRWVEKQVILNCHTEIIVYC
IKK-1    509  CSQ--RTEPESSESLRRRPS-----RDQVRE-DRSIOQQLCCLDNHNEIIVKQ
IKKα     521  YAEVGVGYLEHDSLSLHAEK-----QKSEVGRQGLIMSTLEORATDLYKQ
IKKβ     524  CGRENDEKLVLRHMLQKQKTH-----QRSENG-RKQGGTDLLEEQARCTEPR

NAK      566  FRKDKAKRRLAYNEI-Q--LHFDKQKLYYHATKWHYTFDDEGKRYTEKNSSEEWK
IKK-1    557  FRKSRMRPGLGYNEI-Q--LHFDKQKLYYHATKWHYTFDDEGKRYTEKNSSEEWK
IKKα     571  LRKSRSPHNSYSDSTSMVKKKLEHTEQSMHVEKKEFGNLSKLQCKKRTDILPRVEVAT
IKKβ     574  LREKPRDRTEGDSQEMVRAKQAKQSFRKKRVAVYTLQSKTVVGRKAKALELPRVEEVV

NAK      621  RMYLHLRNGQKLSFNQCFDIEPEKSYQCEYT--NELOEYLP---QKMPASG-GIKH
IKK-1    612  RMYHGTNRHRLGCSVAACNDEAGQKQSL--SKLLEEES---HQLLQDRKAGAOAM
IKKα     630  SMNKEADNIVFMKQGRKREWHLLKIACQSSARSIVGSSSLGAVTPTQTSWLPPTSAE
IKKβ     634  SMINEDEKLVWRLOEKROKESMNLKTIACKK--VGGPVVGGSPD--SMNASRLRQPGGLM

NAK      673  MTP-----IYFS-SNLLVEMTLEGGKLRKREKGVKRETAEN-NHIEPFGSLTMDGGLRN
IKK-1    665  PFP-----IAPYFPPPRKQKLEHMOELCGMKLCASTLRDN-NRIERLNRVPAPOV--
IKKα     690  HDHSLSCVYTPQDGETSADMTPELNLCLGHISTTITHEANEEQGNMNMNLDWSWLTE---
IKKβ     689  SQPSTASNSLPEPKKKSPEKVAEARRLCTLLENAQDQTYREERQSFATALDWSWLTEERE

NAK      726  VDCL-----
IKK-1    -----
IKKα     -----
IKKβ     749  HSCLEQAS

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