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Signal Transduction Research Antibodies

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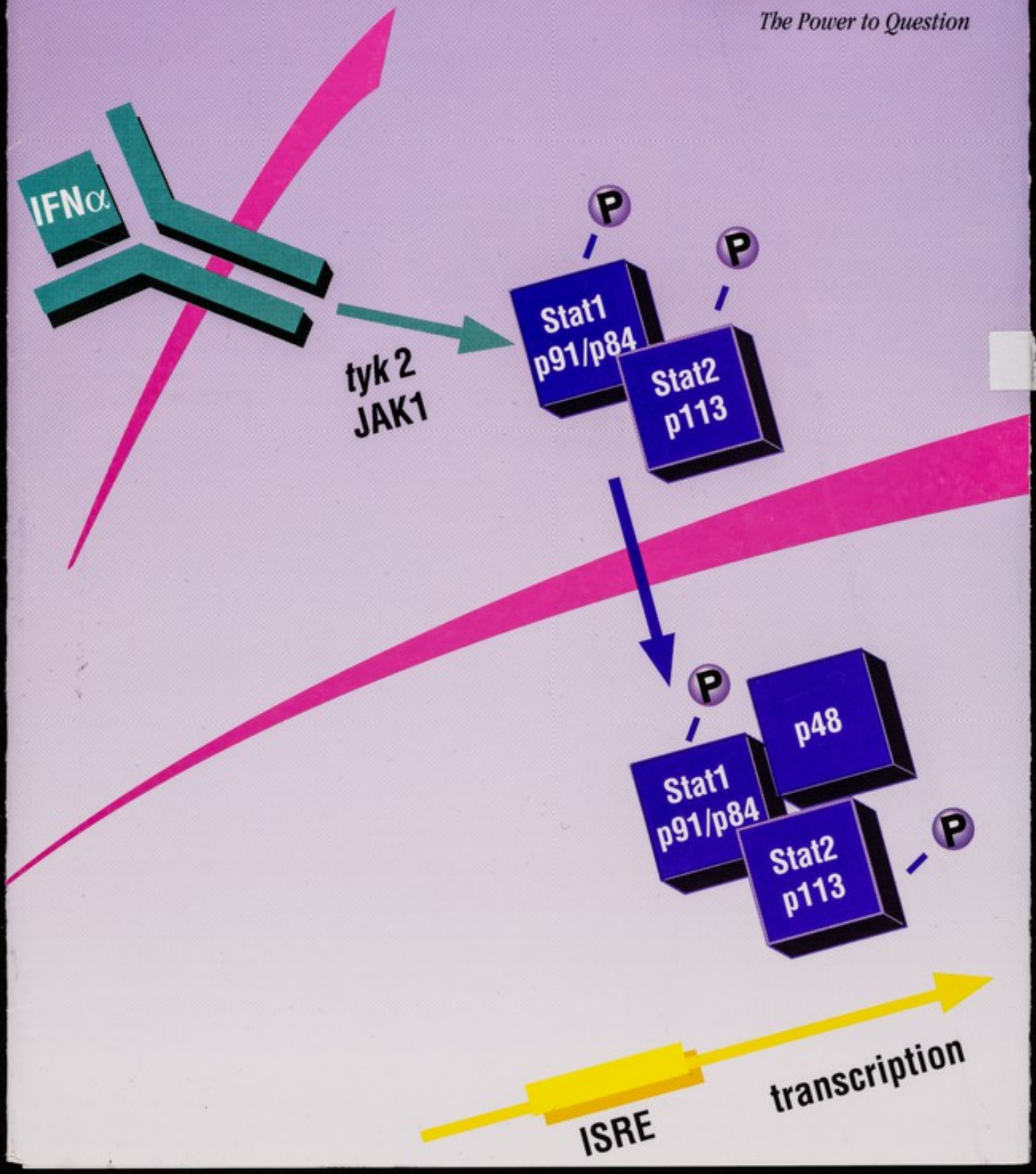
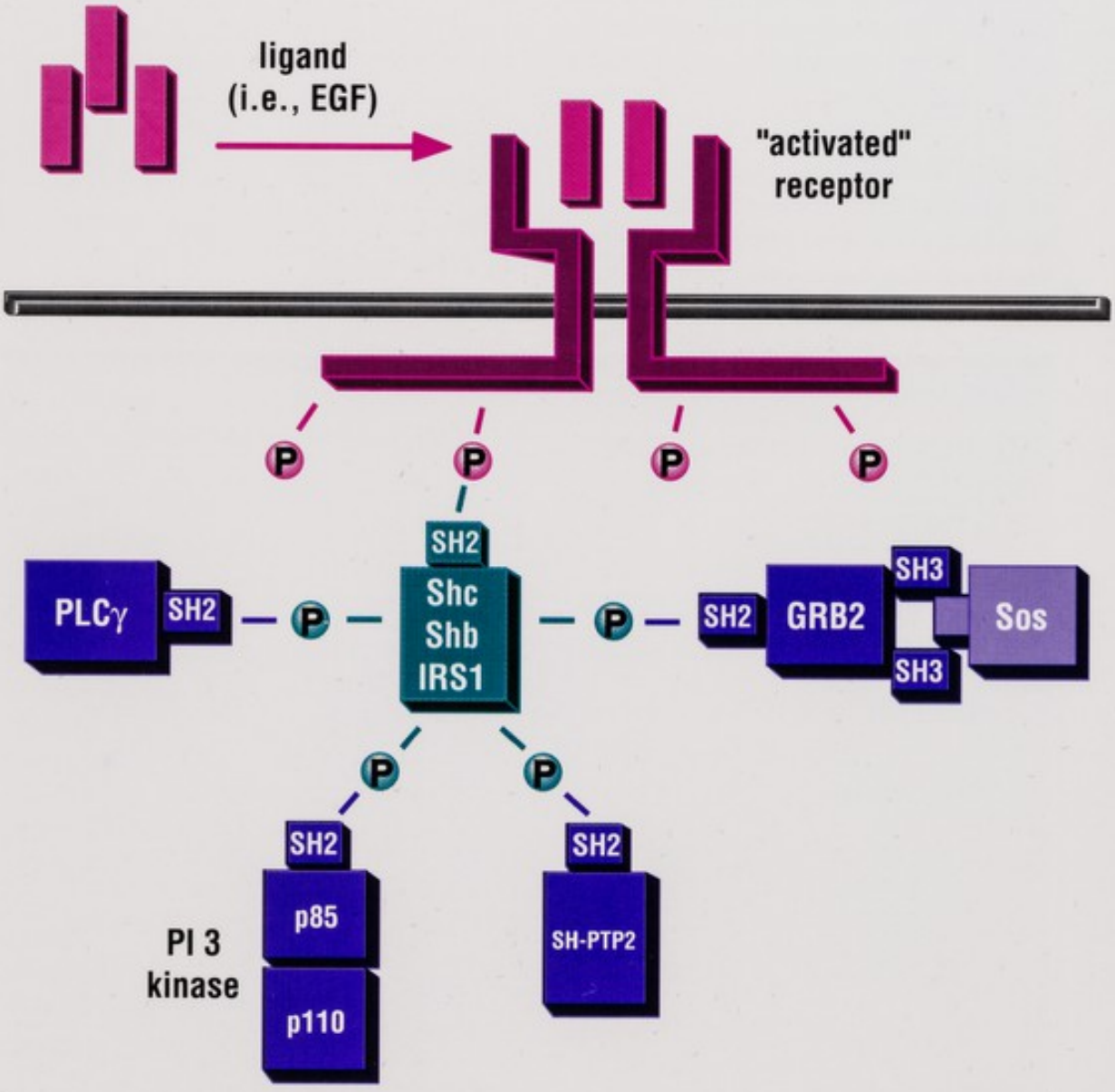
The Power to Question



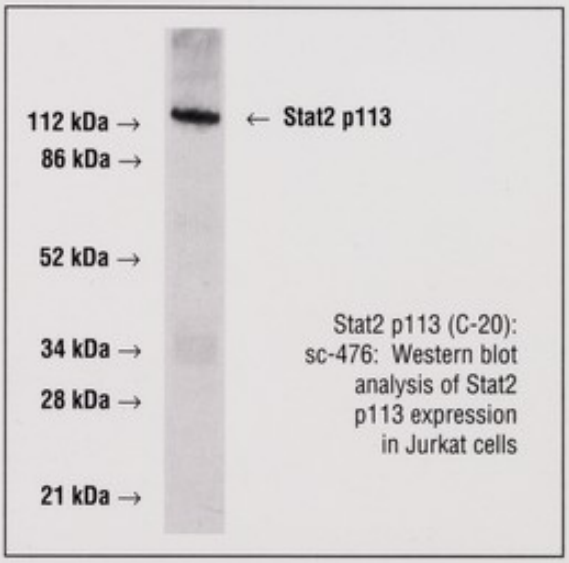
Cell Signaling Pathways

Signaling from ligand-activated membrane receptors to the nucleus involves multiple, pathways functioning in concert to dictate cellular responses. The best studied of these include the JAK/Stat and *ras*/MAP kinase pathways (1). Insight into the JAK/Stat pathway was initially derived from studies of the interferon signaling pathways. For example, the binding of interferon α to its receptor results in the activation of two tyrosine protein kinases, JAK1 and *tyk* 2. Subsequently, these kinases phosphorylate specific tyrosine residues of the Stat1 (either Stat1α p91 or Stat1β p84) and Stat2 (p113) transcription factors. The Stat 1 and 2 transcription factors form heterodimeric complexes which relocate to the nucleus, bind a protein designated p48, and bind to the ISRE DNA element to activate transcription of interferon α regulated target genes (1).



A broad range of ligand-induced responses are mediated via the JAK/Stat pathway. These extend not only to interferons and hematopoietic factors but also to growth factors specifically targeted to protein tyrosine kinase membrane receptors. To accommodate signaling from these diverse ligand activated receptors, diversity within the JAK/Stat signaling pathway is required. For instance, multiple members of the JAK family of protein kinases have been described, including JAK1, JAK2, JAK3, and *tyk* 2 (2–4). Similarly, the Stat family of transcription factors identified to date is comprised of multiple members, including Stat1α, 1β, 2, 3, 4, 5, and IL-4 Stat (5–8), each of which can differentially regulate transcription through the formation of various combinations of homo- and heterodimeric complexes (1). Further diversity is derived through interactions with p48, and the related interferon response factor-1 (IRF-1) and IRF-2 proteins, that act to modify Stat transcription factor activity (9, 10).

The second well-studied signaling pathway involves activation of the MAP kinase phosphorylation cascade as a consequence of protein tyrosine kinase membrane receptor activation. Various growth factor ligands such as EGF, bind their cognate membrane receptors, induce receptor subunit dimerization and transphosphorylation of critical tyrosine residues mapping within the intracellular domains of the receptor subunits. These phosphotyrosine residues, in turn, bind SH2 domain's of various signaling intermediates including those with catalytic activity such as PLC γ, PI 3 kinase, SH-PTP2 (*Syp*), and certain members of the JAK kinase family. Other signaling intermediates such as GRB2, GRB3 (*crk*) and GRB4 (*nck*), function to facilitate protein-protein interactions but themselves lack catalytic activity.



Stat2 p113 (C-20): sc-476: Western blot analysis of Stat2 p113 expression in Jurkat cells

JAK/STAT SIGNALING PROTEINS

product	cat. #	isotype	applications	species
tyk 2 (C-20)	sc-169	rabbit IgG	WB, IP, IHC	mouse, rat, human
JAK1 (Q-19)	sc-295	rabbit IgG	WB, IP, IHC	mouse, rat, human
JAK2 (C-20)	sc-294	rabbit IgG	WB, IP, IHC	mouse, rat, human
JAK3 (C-21)	sc-513	rabbit IgG	WB, IP, IHC	mouse, rat, human
Stat1 p91 (C-111)	sc-417	mouse IgG_1	WB, IP, IHC, GS	mouse, rat, human
Stat1 p91 (C-24)	sc-345	rabbit IgG	WB, IP, IHC, GS	mouse, rat, human
Stat1 p84/p91 (C-136)	sc-464	mouse IgG_1	WB, IP, IHC, GS	mouse, rat, human
Stat1 p84/p91 (E-23)	sc-346	rabbit IgG	WB, IP, IHC, GS	mouse, rat, human
Stat2 p113 (C-20)	sc-476	rabbit IgG	WB, IP, IHC, GS	mouse, rat, human
Stat3 (K-15)	sc-483	rabbit IgG	WB, IP, IHC, GS	mouse, rat, human
Stat3 (C-20)	sc-482	rabbit IgG	WB, IHC, GS	mouse, rat, human
Stat4 (L-18)	sc-485	rabbit IgG	WB, IP, IHC, GS	mouse, rat, human
Stat4 (C-20)	sc-486	rabbit IgG	WB, IP, IHC, GS	mouse, rat, human
IRF-1 (C-20)	sc-497	rabbit IgG	WB, IHC, GS	mouse, rat, human
IRF-2 (C-19)	sc-498	rabbit IgG	WB, IHC, GS	mouse, rat, human
ISGF-3γ p48 (C-20)	sc-496	rabbit IgG	WB, IHC, GS	mouse and rat

WB - Western blotting, IP - Immunoprecipitation, IHC - Immunohistochemistry, GS - Gel Supershift

SH2/SH3 SIGNALING INTERMEDIATES

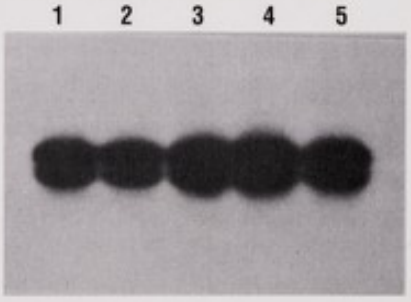
product	cat. #	isotype	applications	species
GRB2 (C-23)	sc-255	rabbit IgG	WB, IP, IHC	mouse, rat, human
GRB2 (217)	sc-422	rabbit IgG	WB, IP, IHC	mouse, rat, human
GRB2 (1-68)	sc-503	mouse IgG	WB, IP, IHC	mouse, rat, human
vav (C-14)	sc-132	rabbit IgG	WB, IP, IHC	mouse, rat, human
ZAP-70 (485)	sc-157	rabbit IgG	WB, IP, IHC	mouse, rat, human
ZAP-70 (LR)	sc-574	rabbit IgG	WB, IP, IHC	mouse, rat, human
syk (LR)	sc-573	rabbit IgG	WB, IP, IHC	mouse, rat, human
Sbc (C-20)	sc-288	rabbit IgG	WB, IP, IHC	mouse, rat, human
csk (C-20)	sc-286	rabbit IgG	WB, IP, IHC	mouse, rat, human
ctk (C-20)	sc-470	rabbit IgG	WB, IP, IHC	mouse and rat
lck (3A5)	sc-433	mouse IgG_{2b}	WB, IP, IHC	mouse, rat, human
lck (2102)	sc-13	rabbit IgG	WB, IP, IHC	mouse, rat, human
c-*yes* (3)	sc-14	rabbit IgG	WB, IP, IHC	mouse, rat, human
lyn (44)	sc-15	rabbit IgG	WB, IP, IHC	mouse, rat, human
c-*src* (N-16)	sc-19	rabbit IgG	WB, IP, IHC	mouse, rat, human
fyn (FYN3)	sc-16	rabbit IgG	WB, IP, IHC	mouse, rat, human
fyn (15)	sc-434	mouse IgG_1	WB, IP, IHC	mouse, rat, human
bck (N-30)	sc-72	rabbit IgG	WB, IP, IHC	mouse, rat, human

WB - Western blotting, IP - Immunoprecipitation, IHC - Immunohistochemistry

The binding of signaling molecules, such as GRB2, to receptors is either direct, or indirect. Indirect binding is mediated either by protein tyrosine phosphatases (11, 12) or by a class of proteins of which *Sbc, Sbb,* (13, 14) and the insulin receptor substrate-1, IRS-1, (15, 16) proteins represent prototypes. These latter proteins bind activated membrane receptors through their SH2 domains and are themselves phosphorylated on tyrosine residues to which the various signaling intermediates then bind (13–16). In addition to the broad family of transmembrane protein tyrosine kinase receptors, a second class of protein tyrosine kinases are intracellular but function in a manner analogous to the intracellular domains of the transmembrane receptors. These latter kinases include members of the *src* gene family and functionally-related proteins such as ZAP-70 and *syk* (17, 18).

In its activated form, GRB2 binds the guanine nucleotide exchange protein, Sos, through the binding of its SH3 domain to Sos proline rich sequences. Sos mediates *ras* protein activation by facilitating exchange of GDP for GTP, and *ras*, in turn, activates both MEK kinase (19) and *raf* which translocates to the cell membrane (20, 21), binds 14–3–3 (22, 23) and, in its activated form, functions as a MEK kinase converting MEK from its inactive to activated form through phosphorylation at ser-218 and ser-222 (24, 25). In addition, *ras* has recently been shown to interact directly with the catalytic subunit of PI 3 kinase in a GTP-dependent manner (26).

Activated MEK, also designated MAP kinase kinase, functions to activate MAP kinases (ERK proteins) via



lyn (44): sc-15: immune complex protein tyrosine kinase analysis of *lyn* p53/p56 expression in RBL-2H3 cells

phosphorylation at a tyr-183/thr-185 regulatory motif. In turn MAP kinases phosphorylate the *elk* transcription factor which upregulates transcription of *fos* (27). A MAP kinase related protein designated *jun* kinase-1, or JNK 1, also activated by phosphorylation of a tyr-183/thr-185 motif, functions to activate a second member of the AP-1 transcription factor family, *jun*, via phosphorylation of ser 63 and ser 73 residues (28–30) while *fos* regulating kinase (FRK) functions to activate *fos* via phosphorylation of thr-232 (31). An additional MAP kinase related protein, p38, with a similar tyr/thr phosphorylation regulatory motif, has been identified as the mammalian homolog of the yeast protein, HOG-1 (32).

Cellular responses to ligand activation including differentiation, mitotic stimulation/arrest, or apoptosis are ultimately determined by the balance of signaling between diverse pathways such as those described above. Much remains to be done to further map these pathways and to delineate their role in cell signaling.

MAP KINASE SIGNALING PATHWAY

product	cat. #	isotype	applications	species
v-H-*ras* (259)	sc-35	rat IgG_1	WB, IP, IHC	mouse, rat, human
ras GAP (B4F8)	sc-63	mouse IgG_{2a}	WB, IP, IHC	mouse, rat, human
raf-1 (C-20)	sc-227	rabbit IgG	WB, IP, IHC	mouse, rat, human
raf-A (C-20)	sc-408	rabbit IgG	WB, IP, IHC	mouse, rat, human
raf-B (C-19)	sc-166	rabbit IgG	WB, IP, IHC	mouse, rat, human
MEK kinase (C-22)	sc-252	rabbit IgG	WB, IP, IHC	mouse, rat, human
MEK kinase (1-9C-2A)	sc-449	mouse IgG_1	WB, IP	human specific
MEK-1 (12-B)	sc-436	rabbit IgG	WB, IP, IHC	mouse, rat, human
MEK-1 (C-18)	sc-219	rabbit IgG	WB, IHC	mouse, rat, human
MEK-2 (C-16)	sc-525	rabbit IgG	WB, IP, IHC	mouse, rat, human
ERK 1 (C-16)	sc-93	rabbit IgG	WB, IP, IHC	mouse, rat, human
ERK 1 (K-23)	sc-94	rabbit IgG	WB, IHC	mouse, rat, human
ERK 2 (C-14)	sc-154	rabbit IgG	WB, IP, IHC	mouse, rat, human
JNK 1 (N-19)	sc-473	rabbit IgG	WB, IP, IHC	mouse, rat, human
JNK 1 (C-17)	sc-474	rabbit IgG	WB, IP, IHC	mouse, rat, human

AP-1 TRANSCRIPTION FACTORS

product	cat. #	isotype	applications	species
c-*fos* (4)	sc-52	rabbit IgG	WB, IHC, GS	mouse, rat, human
c-*fos* (K-25)	sc-253	rabbit IgG	WB, IP, IHC, GS	mouse, rat, human
c-*fos* (4-10G)	sc-413	mouse IgG_{2a}	WB, IP, GS	mouse, rat, human
fos B (102)	sc-48	rabbit IgG	WB, IP, GS	mouse, rat, human
c-*jun*/AP-1 (D)	sc-44	rabbit IgG	WB, IP, GS	mouse, rat, human
c-*jun*/AP-1 (N)	sc-45	rabbit IgG	WB, IP, IHC, GS	mouse, rat, human
jun B (N-17)	sc-46	rabbit IgG	WB, IP, IHC, GS	mouse, rat, human
jun D (329)	sc-74	rabbit IgG	WB, IP, IHC, GS	mouse, rat, human

WB - Western blotting, IP - Immunoprecipitation, IHC - Immunohistochemistry, GS - Gel Supershift

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nine kinases

, PKC (α, βI, βII, γ, θ, δ, ase p85, p110, MAP kinase, EK kinase and JNK1

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- **transcription factors**

  NFκB p65, p50, p52, rel, rel B, IκB-α/MAD-3, WT, E2F-1, E2F-4, Sp1, AP-2, YY1, oct-1, oct-2, Max, Mad, CREB, ATF (1-4), TFIIB, TFIID, GAL4 (DBD), GAL4 (TA), maf and NF-E2

- **GTP binding proteins**

  H-ras, N-ras, K-ras, rho A, rho B, rab 1, rab 2, rab 3A, rab 3B, rab 4, rab 5A, rab 6, rab 8, rap 1, rap 2, rac 1, rac 2, rho GDI, heterotrimeric G protein α, β and γ subunits

- **serine/threonine kinases**

  raf-1, raf A, raf B, PKC (α, βI, βII, γ, θ, δ, ε, η, ζ), PI 3 kinase p85, p110, MAP kinase, MEK-1, MEK-2, MEK kinase and JNK1

- **signaling intermediates**

  GRB2, vav, Sos, nck, crk, crk-L, dbl, ras GAP, ras-GRF, and PLC (γ1, γ2, β1, β2, β3, β4, δ1, δ2)

- **muscle-specific transcripton factors**

  Myo D, myogenin, myf-5, myf-6, MEF-2, xMEF-2, HEB, E12, E47 and Id (1-4)

- **tumor suppressors**

  Rb p110, p107, p130, WAF1/Cip1 p21, p53, p27, MTS1 p16 and hMSH2

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