

BACKGROUND

The p21-activated kinases (Paks) are Ser/Thr protein kinases that bind to and, in some cases, are stimulated by activated forms of the small GTPases, Cdc42 and Rac. Paks are categorized into two subgroups based on architectural similarities. The conventional Group I Paks (Pak1, Pak2, Pak3) and nonconventional Group II Paks (Pak4, Pak5, Pak6). PAKs are stimulated dramatically by the GTP-bound forms of small GTPases, but also by a variety of GTPase-independent mechanisms. PAKs have an ancient origin and serve as important regulators of cytoskeletal dynamics and cell motility. In addition, they are implicated in transcription through MAPK cascades, death and survival signaling, and cell-cycle progression. Consequently, PAKs are implicated in a number of pathological conditions and in cell transformation. Paks function as downstream nodes for various oncogenic signaling pathways. Paks have also been shown to promote cell proliferation and accelerate mitotic abnormalities, which results in tumor formation and cell invasiveness.¹

The conventional PAK family contains a conserved Cdc42/Rac-interacting binding domain (CRIB) that overlaps a kinase-inhibitory (KI) domain. There are three conserved proline-rich motifs that bind SH3 domain-containing proteins Nck, Grb2 and PIX. The group II PAKs (PAK4-PAK6) contains a CRIB sequence that binds GTPases, but lacks a KI domain. Upon Cdc42 (or related GTPase) binding, proteolysis or lipid binding, the kinase undergoes a conformational change that allows autophosphorylation. Phosphorylation of Ser-144 serves to disable the KI-domain-kinase interaction, while phosphorylation of Ser-198/203 reduces the affinity for PIX. Phosphorylation of the activation-loop Thr-422 may occur in *trans*, or may involve a third-party kinase such as PDK1. Other protein kinases might down-regulate PAK function: Akt phosphorylates PAK1 at Ser-21(Ser20 in PAK2), and this modification decreases binding of Nck to the PAK1 N-terminus while increasing kinase activity, which would suggest inhibition of PAK when Nck recruits PAK to various phosphotyrosine-containing complexes.²

RhoA, Rac1 and Cdc42 interact with a variety of associated kinases. Myosin light chain kinase (MLCK) is a calcium/calmodulin-responsive enzyme that maintains the myosin heavy chain-MLC complex in an active state, but is negatively regulated by PAK. Various studies have implicated ROK, MRCK and PAK in the regulation of LIMKs, which inactivate cofilin by phosphorylation at Ser-3. Once phosphorylated, cofilin/ADF (actin depolymerizing factor) can no longer bind effectively to F-actin, and the ability of these proteins to catalyze both F-actin depolymerization and severing is thus inhibited. PAK1 is thought to modulate R-MLC function primarily via inhibition of MLCK activity. Phosphorylation of MLCK occurs at

Ser-439 and Ser-991; binding of calmodulin to MLCK is inhibited by modification of Ser-991. PAK1 has been shown to be able to bind to and regulate Ser-508 within the LIMK1 activation loop downstream of Rac1; Rho and Cdc42 are more closely linked to the effects of LIMK2. Thus the Rac and Cdc42 signaling pathways, acting via PAKs, can function either co-operatively with or antagonistically to Rho/ROK.³

References:

1. Kumar, R. et al: Nature Rev. Cancer 6:459-71, 2006
2. Daniels, R. & Bokoch, G.M. et al: Trends Biochem. Sci. 24:350-5, 1999
3. Gerthoffer, W.T. & Gunst, S.J.: J. Appl. Physiol. 91:963-72, 2001

TECHNICAL INFORMATION

Source:

PAK2 Antibody is a mouse monoclonal antibody raised against purified recombinant human PAK2 fragments expressed in *E. coli*.

Specificity and Sensitivity:

This antibody detects endogenous PAK2 proteins without cross-reactivity with other family members.

Storage Buffer: PBS and 30% glycerol

Storage:

Store at -20°C for at least one year. Store at 4°C for frequent use. Avoid repeated freeze-thaw cycles.

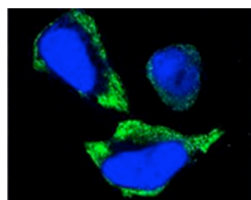
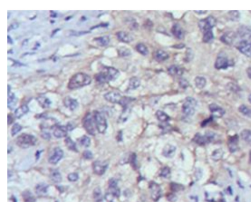
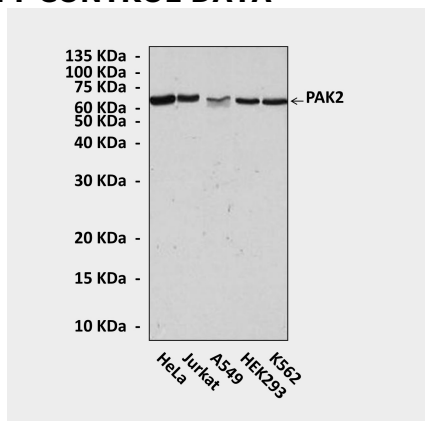
APPLICATIONS

Application:	*Dilution:
WB	1:1,000
IP	1:50
IHC	1:200
ICC	1:200
FACS	n/d

**Optimal dilutions must be determined by end user.*



QUALITY CONTROL DATA



Top: Western Blot detection of PAK2 proteins in various cell lysates using PAK2 Antibody. **Middle:** This antibody stains paraffin-embedded human lung cancer tissue in immunohistochemical analysis. **Bottom:** It also stains HeLa cells in confocal immunofluorescent testing (PAK2 Antibody: Green; DRAQ5 DNA dye: Blue).

