

Certificate of Analysis

anti-PAK3, rabbit monoclonal

(Rabbit monoclonal IgG)

Catalog # 04-395

Lot # 0611045589

Immunogen: KLH-conjugated synthetic peptide corresponding to N-terminus of human PAK3 (p21-Activated Kinase 3).

Specificity: Recognizes the N-terminus of PAK3.

Molecular Weight: 65 kDa.

Entrez Gene Accession #: NP_002569.1

Species Cross-reactivity: Reacts with Human, Mouse, and Rat.

Formulation: 100 μ L of rabbit monoclonal IgG in 30 mM Tris-Glycine (pH 7.4), 0.09 M NaCl, 0.006% sodium azide, 0.03% BSA and 40% glycerol.

Storage and Stability: Stable for 2 years at -20°C from date of shipment.

Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: A 1:10,000-50,000 dilution of the lot detected endogenous PAK3 in fetal brain lysate.

Immunoprecipitation:

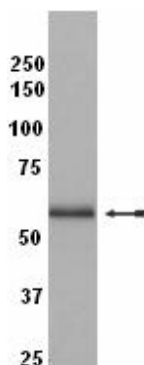
A 1:50 dilution of this antibody immunoprecipitated PAK3 using protein G:agarose beads.

Immunohistochemistry:

A 1:100-1:250 dilution of this antibody detected PAK3 in human brain glioma using immunohistochemical analysis of Paraffin-embedded tissue.

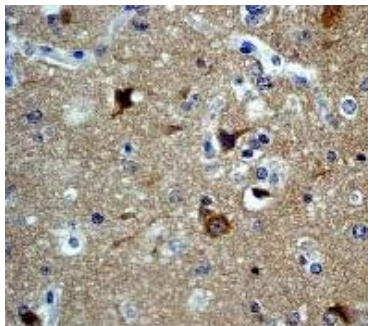
Immunofluorescence Staining:

1:100-1:250 dilution of this antibody was used for immunofluorescence staining of paraformaldehyde fixed cells.



Immunoblot Analysis:

Lysate from fetal brain was resolved by electrophoresis, transferred to PVDF and probed with anti-PAK3 (1:50,000). Proteins were visualized using goat anti-rabbit secondary antibody conjugated to HRP and chemiluminescence detection. Arrow indicates PAK3 (65 kDa).



General References:

1. Boda, Bernadett, *et al* (2004). The mental retardation protein PAK3 contributes to synapse formation and plasticity in hippocampus. *J Neurosci* **24**: 10816-25.
2. Rousseau, Veronique, *et al* (2003). A new constitutively active brain PAK3 isoform displays modified specificities toward Rac and Cdc42 GTPases. *J Biol Chem* **278**: 3912-20.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate samples (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na₃VO₄, 1 mM NaF) and transfer the proteins to PVDF. Wash the blotted PVDF twice with TBST.
2. Block the blotted PVDF in freshly prepared 5% BSA in TBS with 0.05% Tween[®]-20 for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with 1:50,000 dilution of **anti-PAK3**, diluted in freshly prepared 5% BSA in TBST for 2 hours at room temperature or overnight with agitation at 4°C .
4. Wash the PVDF 3 times with TBST.
5. Incubate the PVDF in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in TBST/5% BSA for 1 hour with agitation at room temperature.
6. Wash the PVDF 3-5 times with TBST.
7. Use detection method of choice (enhanced chemiluminescence was used).

Immunofluorescence Staining

1. Fix cells with 3.7% paraformaldehyde for 20 min.
2. Wash cells twice with 1X PBST.
3. Permeabilize cells with 0.1% Triton-X100 for 5 min
4. Wash cells twice with 1X PBST.
5. Block cells with blocking buffer for 1 hour at room temperature (option - O/N 4 °C).
6. Dilute anti-mTOR antibody in the blocking buffer at a 1:50-1:100 dilution and apply on the cells and incubate at room temperature for 2 hours or overnight at 4 °C.
7. Wash cells twice with 1X PBST.
8. Incubate cells in a dilution of fluorescently-labeled secondary antibody in PBS for 45 min at room temperature in the dark.
9. Wash cells three times with 1X PBST.
10. Counterstain cells with DAPI in a concentration of 300 nM in PBS for 5 min in the dark.
11. Wash cells three times with 1XPBST.
12. Mount slides with medium for fluorescent staining.
13. Store slides in the dark.



Rabbit Monoclonals Produced Using Technology from Epitomics, Inc. Under Patent No. 5,675,063