# ProductInformation

**ANTI-PAK3**
**Developed in Rabbit, IgG Fraction of Antiserum**

Product Number **P2979**

## Product Description

Anti-PAK3 is developed in rabbit using a synthetic peptide corresponding to amino acids 1-13 of the N-terminal of mouse p21 activated kinase 3 (PAK3) conjugated to KLH as immunogen. The antibody is purified using protein A chromatography.

Anti-PAK3 recognizes mouse PAK3 (65 kD) by immunoblotting. PAK3 was not detected in mouse brain cytosol. It also reacts with rat PAK3, and weakly with human PAK3.

Anti-PAK3 may be used for immunoprecipitation and immunoblotting of PAK3.

PAK3 is a 65 kD isoform of p21 activated kinase 3. This serine/threonine kinase, which binds to the small G-proteins cdc 42 and Rac, is reported to be the mammalian homolog of Ste 20 kinase in S. cerevisiae. Transfection of Cos cells or insect cells with a cDNA encoding PAK3 was found to activate the 78 kD isoform of MEKK1, although this isoform of MEKK1 does not appear to be direct substrate of PAK3. PAK proteins also appear to be crucial effectors linking rho GTPases to cytoskeletal reorganization and to nuclear signaling. Recently, a point mutation in PAK3 that causes premature termination and loss of kinase function was found in some individuals and nonsyndromic X-linked mental retardation. MRI analysis of affected individuals showed no gross defects in brain development. Immunofluorescence analysis showed that PAK3 is highly expressed in postmitotic neurons of the developing and postnatal cerebral cortex and hippocampus. These results suggest that signal transduction through PAK3 may be critical for human cognitive function.

## Reagents

The product is supplied as IgG fraction in 0.07 M Tris-glycine buffer, pH 7.4, containing 0.105M NaCl, 30% glycerol and 0.035% sodium azide (see MSDS)* as a preservative.

Protein concentration is approximately 0.2 mg/ml by Bradford analysis.

## Precautions and Disclaimer

* Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

## Storage/Stability

Store at 0°C to –20°C. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

## Procedure

1. Dilute the cell lysate before beginning the immunoprecipitation to roughly 1µg/µl total cell protein in a microcentrifuge tube with PBS (Sigma Product No. P3813).
2. Add 4 µg of anti-PAK3 to 500 µg - 1mg cell lysate.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 µl of a washed (in PBS) 1:1 slurry of Protein A-Agarose beads (50 µl packed beads) (Sigma Product No. P2545).
5. Gently rock reaction mixture at 4°C for 2 hours.
6. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice cold cell lysis buffer or PBS.
7. Resuspend the agarose beads in 50 µl 2X Laemmli sample buffer. The agarose beads can be frozen for later use.
8. Suspend the agarose beads in Laemmli sample buffer and boil for 5 minutes. Pellet the beads using a microcentrifuge pulse. SDS-PAGE and subsequent immunoblotting analysis may be performed on a sample of the supernatant.

Lysis Buffer:
50 mM Tris-HCl, pH 7.4, containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA, 1 mM PMSF, 1 µg/ml each aprotinin, leupeptin, pepstatin, 1 mM $Na_3VO_4$, and 1 mM NaF.

**Product Profile**
Recommended use: 4 µg of Anti-PAK3-1 will immunoprecipitate PAK3 from 0.5-1 mg of a cell lysate of mouse NIH 3T3 cells. Recommended working concentration is 1-2 ug/ml of Anti-PAK3 by immunoblotting using a mouse NIH 3T3 cell lysate, anti-rabbit IgG conjugated to peroxidase and enhanced chemiluminescence.

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

**General Reference**
Allen, K.M., et al., Nat. Genet., **20 (1)**, 25 (1998).

lpg 4/99