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ProductInformation

Anti-phospho-PAK1/2/3 [pSer^{141}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **P 7871**

Product Description

Anti-phospho- PAK1/2/3 (P21-activated kinase) [pSer^{141}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human PAK2 that contains serine 141 as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated PAK2 peptide.

The antibody detects human PAK1 (46% homologous), PAK2, and PAK3 (46% homologous). Other species have not been tested. It has been used in immunoblotting applications.

P21-activated kinase (PAK) is actually a family of serine/threonine protein kinases, members of which are activated by small molecular weight GTPases. The three most common isoforms are PAK1, PAK2, and PAK3 (also known as □α-PAK, γ-PAK, and β-PAK, respectively). These kinases contain numerous regulatory elements that trigger diverse signaling processes such as those initiated by activated GTPases, interaction with Src homology 3 (SH3) domains, and caspase mediated proteolytic cleavage.

Autophosphorylation of serine 141 (serine 144 for PAK1 and serine 139 for PAK3), catalyzed by Cdc42, is required for activation of PAK.

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 50% glycerol, 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide

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 hazards and safe handling practices.

Storage/Stability

Store at –20 °C. For continuous use, store at 2-8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours..

Product Profile

The supplied reagent is sufficient for 10 immunoblots.

A recommended working dilution of 1:1000 is determined by immunoblotting using Cos cells transiently transfected with PAK1, PAK2, or PAK3, and co-transfected with V12 Cdc42 and grown either as adherent cultures to activate PAK, or placed in suspension to suppress PAK activation.

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

Results

Peptide Competition

1. Extracts prepared from Cos cells transiently transfected with PAK1 (lanes 1-4), PAK2 (lanes 5-8), or PAK3 (lanes 9-12) and co-transfected with V12 Cdc42 to induce activation were resolved by SDS-PAGE on a 10% polyacrylamide gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:
   Lane 1, 5, 9 no peptide
   Lane 2, 6, 10 non-phosphorylated peptide corresponding to the immunogen
   Lane 3, 7, 11 a generic phosphoserine containing peptide
   Lane 4, 8, 12 immunogen

4. After preincubation membranes were incubated with 0.50 µg/mL PAK1/2/3 [pSer^{141}] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase and signals were detected.

The data in Figure 1 show that PAK1/2/3 [pSer^{141}] antibody is highly selective for the phosphorylated form of PAK, and that the antibody can be used to detect an active form of PAK1 (see above left), as well as PAK2 and PAK3 (data not shown).

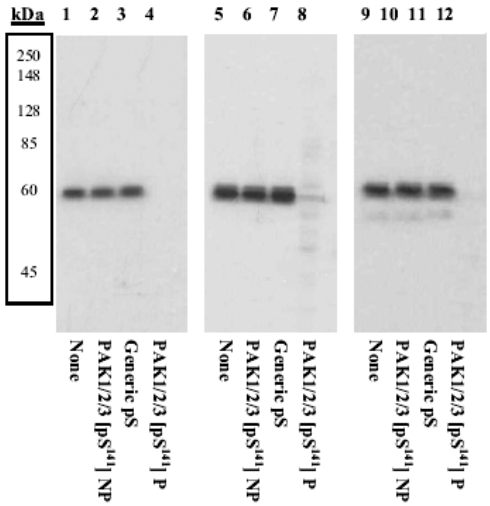

Figure 1 Peptide Competition

References

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