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Product Information

Anti-phospho-β-Arrestin-1 [pSer^{412}]
affinity isolated antibody, aqueous solution

Catalog Number **A8728**

Product Description

Anti-phospho-β-arrestin-1 [pSer^{412}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of rat β-arrestin-1 that contains Ser^{412} as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated β-arrestin-1 peptide.

The antibody detects mouse and rat β-arrestin-1. Human and cow species have not been tested, but due to the high degree of homology (77%) are expected to cross react. It has been used in immunoblotting applications.

β-arrestin-1 is a member of a family of proteins widely expressed but especially abundant in the central nervous system. Serving as an adaptor or scaffold molecule, β-arrestin-1 is essential for mitogenic signaling and mediates agonist-dependent desensitization and internalization of G protein-coupled receptors (GPCRs, e.g., β_2-adrenergic receptor). After binding to their ligand and interacting with heterotrimeric G proteins, GPCRs are phosphorylated by G-protein receptor kinases (GRKs) on serine residues.

β-arrestin-1 in the cytosol is phosphorylated by ERK1&2 on Ser^{412} in a negative feedback mechanism and binds to the phosphorylated receptors at the plasma membrane. Ser^{412} is then dephosphorylated and the GPCRs are internalized, leading to activation of the Ras → Raf → ERK1&2 signaling pathway.

Reagent

Anti-phospho-β-arrestin-1 [pSer^{412}] is provided as a solution in Dulbecco's PBS, pH 7.3 with 1 mg/mL BSA and 0.05% Sodium Azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at –70 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 blots.

A recommended working concentration of 0.1 to 1.0 μg/mL is determined by immunoblotting using PC12 and CHO-K cells.

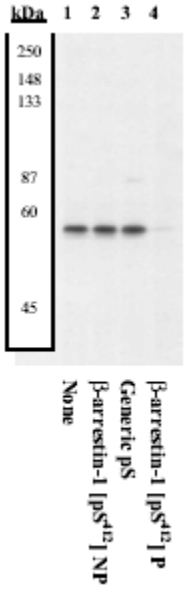
Note: In order to obtain best results in different techniques and preparations, it is recommended to determine optimal working concentration by titration test.

Results

Peptide Competition

1. Extracts prepared from PC12 cells were resolved by SDS-PAGE on a 10% Tris-glycine 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 no peptide
   - Lane 2 non-phosphorylated peptide corresponding to the immunogen
   - Lane 3 a generic phosphoserine containing peptide
   - Lane 4 immunogen
4. After preincubation membranes were incubated with 0.75 μg/mL β-arrestin-1 [pSer^{412}] 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.

Figure 1.
Peptide Competition



The data in Figure 1 show that only the peptide corresponding to β-arrestin [pSer^{412}] blocks the antibody signal, thereby demonstrating the specificity of the antibody.

References

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2. Miller, W.E. et al., beta-arrestin1 interacts with the catalytic domain of the tyrosine kinase c-SRC. Role of beta-arrestin1-dependent targeting of c-SRC in receptor endocytosis. J. Biol. Chem., **275**, 11312-11319 (2000).
3. Lefkowitz, R.J., G protein-coupled receptors. III. New roles for receptor kinases and beta arrestins in receptor signaling and desensitization. J. Biol. Chem., **273**, 18677-18680 (1998).

SG,AH,JK,MAM 05/18-1