

**ANTI-PHOSPHATIDYLINOSITOL 3-KINASE**

**(PI 3-Kinase, p85 $\alpha$ )**

**Developed in Rabbit**

**Delipidized, Whole Antiserum**

Product No. **P8208**

Lot 105H4851

Anti-Phosphatidylinositol 3-Kinase (PI 3-Kinase, p85 $\alpha$ ) is developed in rabbit using a synthetic peptide (Asn-Asp-Ser-Leu-Asn-Val-Thr-Leu-Ala-Tyr-Pro-Val-Tyr-Ala-Gln-Gln-Arg-Arg) corresponding to the C-terminal region (SH2 domain, amino acids 707-724) of the p85 $\alpha$  regulatory subunit of PI 3-kinase coupled to KLH as immunogen. This sequence is highly conserved among species and is identical in human, bovine, mouse p85 $\alpha$ , but different from the C-terminal region of the p85 $\beta$  and p110 subunits of PI 3-kinase. The antiserum has been treated to remove lipoproteins and is provided as whole antiserum containing 0.1% sodium azide (see MSDS)\* as a preservative.

**Specificity**

Anti-PI 3-Kinase reacts in immunoblotting (SDS-PAGE) with p85 $\alpha$  subunit (85 kD protein) in rat brain and COS cells extracts. The antiserum may stain an additional band of 46 kD due to non-specific absorption of rabbit immunoglobulins. Staining of the p85 band is inhibited with PI 3-kinase peptide. Anti-PI 3-Kinase reacts in dot blot immunoassay with PI 3-kinase peptide (707-724), conjugated to BSA.

**Protein Concentration:** 67 mg/ml by biuret.

**Titers**

1. A titer of 1:2,000 was determined by indirect immunoblotting using COS cells extract. Specific staining of PI 3-kinase (p85 $\alpha$ ) (85kD band) is observed. PI 3-kinase band is blocked by incubating prediluted antibody with PI 3-kinase peptide (concentration 1.0 $\mu$ g/ml), for 2 hours at room temperature or overnight at 4 $^{\circ}$ C.
2. A titer of 1:1,000 was determined by indirect immunoblotting using rat brain extract. Specific staining of PI 3-kinase (p85 $\alpha$ ) (85kD band) is observed.
3. A titer of 1:16,000 was determined by dot blot immunoassay using PI 3-kinase peptide conjugated to BSA (conjugate concentration 125 ng/dot).

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

**Description**

Phosphatidylinositol 3-kinase (PI 3-kinase), is a phosphoinositide-specific protein kinase that plays a central role in mitogenic signal transduction leading to cellular regulation, cell growth and differentiation, and oncogenesis.<sup>1,2</sup> PI 3-kinase associates with, and is activated by a wide range of tyrosine kinase receptors including EGF, PDGF, CSF-1, erbB-3 and insulin receptor, non-receptor protein-tyrosine kinases of the src family (e.g. pp60<sup>src</sup>), and *crk* and *abl* proto-oncogene products.<sup>1,2,3</sup> PI 3-kinase is involved in signal transduction in terminally differentiated, activated cells like platelets and neutrophils.<sup>4</sup> PI 3-kinase becomes highly phosphorylated on tyrosine residues and activated upon mitogenic stimulation or transformation of cells. Activated PI 3-kinase phosphorylates at the D3 position of the inositol ring of phosphatidylinositol (PI), PI-4-phosphate and PI-4,5-biphosphate, to generate the respective PI 3-phosphorylated derivatives. PI 3-kinase has a heterodimeric structure, consisting of a regulatory 85kD (p85) subunit and a catalytic 110 kD (p110) subunit.<sup>2,5-8</sup> In addition two distinct forms of p85 subunit have been described: p85 $\alpha$  and p85 $\beta$ .<sup>2</sup> The p85 $\alpha$  regulatory subunit associates with ligand-activated receptors, and regulates the catalytic subunit p110. The role of the p85 $\beta$  subunit is largely unknown. The regulatory p85 $\alpha$  subunit contains two SH2 (*src*-homology 2) domains and one SH3 (*src*-homology 3) domain. It has been shown that p85 $\alpha$  associates directly with phosphorylated receptors via the SH2 domain, and is bound tightly to the p110 catalytic subunit.<sup>1,3,9</sup> PI 3-kinase does not directly associate with the insulin receptor, but with one of its tyrosine-phosphorylated substrates IRS-1, which mediates the complex formation.<sup>10</sup> PI 3-kinase directly interacts and is activated by Ras.<sup>11</sup> Antibodies that react specifically with PI 3-kinase subunits are useful for the

study of the specific activation requirements, differential tissue expression as well as intracellular localization of PI 3-kinase.

### Uses

Anti-PI 3-kinase may be used to detect PI 3-kinase in immunoblotting using various cell culture extracts and brain tissue extracts. The antibody may be used to detect PI 3-kinase using chemiluminescence detection systems.

### Storage

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.

\* Due to the sodium azide content a material safety 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.

### References

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