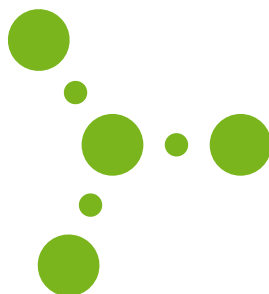


# *PI 3-Kinase Family*



- ▶ *Alpha isoform*
- ▶ *Beta isoform*
- ▶ *Gamma isoform*
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- ▶ *Adaptor Proteins*
- ▶ *Substrates*
- ▶ *Inhibitors*
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- ▶ *PI3 Kinase Assays*



# Jena Bioscience

## Company Profile

Jena Bioscience GmbH was founded by a team of scientists from the Max-Planck-Institute for Molecular Physiology in Dortmund. 25+ years of academic know how were condensed into the company in order to develop innovative reagents and technologies for the life science market.

Since the start up in 1998, the company has evolved into an established global reagent supplier with more than 3000 products on stock and a customer base in 50+ countries. Jena Bioscience serves three major client groups:

- Research laboratories at universities, industry, government, hospitals and medical schools
- Pharmaceutical industry in the process from lead discovery through to pre-clinical stages
- Laboratory & diagnostic reagent kit producers and re-sellers

Our company premises are located in the city of Jena / Germany with a subsidiary in Teltow, just in the vicinity of the German capital Berlin.

Jena Bioscience's products include nucleotides and their non-natural analogs, recombinant proteins & protein production systems, reagents for the crystallization of biological macromolecules and tailor-made solutions for molecular biology and biochemistry.

In our chemistry division, we have hundreds of natural and modified nucleotides available on stock. In addition, with our pre-made building blocks and in-house expertise we manufacture even the most exotic nucleotide analog from mg...kg scale.

In the field of recombinant protein production, Jena Bioscience has developed its proprietary LEXSY technology. LEXSY (Leishmania Expression System) is based on a S1-classified unicellular organism that combines easy handling with a full eukaryotic protein folding and modification machinery including mammalian-like glycosylation. LEXSY is primarily used for the expression of proteins that are expressed at low yields or inactive in the established systems, and expression levels of 300 mg/L of culture were achieved.

For the crystallization of biological macromolecules – which is the bottle neck in determining the 3D-structure of any protein – we offer specialized reagents for crystal screening, crystal optimization and phasing that can reduce the time for obtaining high quality crystals ready for X-ray diffraction from several years to a few days.

Our specialized reagents are complemented with a large selection of products for any molecular biology & biochemistry laboratory such as kits for Standard PCR and Real-Time PCR, oligonucleotides, cloning enzymes, mutagenesis technologies, and many more...

We combine highest quality standards for all our products with an individualized customer support. We establish direct lines of communication from clients to our in-house scientists, resulting in productive interactions among people with similar background and research interest who speak the same language. Furthermore, we offer support programs and attractive discount schemes for young scientists establishing their own labs. If you wish to receive more information on Jena Bioscience, just send us an e-mail to [info@jenabioscience.com](mailto:info@jenabioscience.com).





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# PI 3-Kinase Family

## Introduction

Phosphoinositide 3-kinases (PI3Ks) are a family of related enzymes that play a pivotal role in important cellular regulatory mechanisms. PI3Ks are capable of phosphorylating the 3'-OH position of phosphoinositide lipids (PIs) generating lipid second messengers. Their function has been linked to the regulation of numerous biological processes including cell growth, differentiation, survival, proliferation, migration. On the basis of structural similarities and substrate specificity, the PI3K family is divided into three classes termed I, II, and III.

All human class I members are heterodimers consisting of a catalytic subunit (MW approx. 110 kDa) and a non-catalytic subunit (MW 50, 55, 85, 87 or 101 kDa). They are known to phosphorylate phosphatidylinositol (PI), phosphatidylinositol-4-mono-phosphate (PIP) and phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>) in vitro but have a strong preference for PIP<sub>2</sub> in vivo. Class I members are further subdivided into class IA and IB PI3Ks. Class IA consists of three isoforms (p110α, p110β and p110δ) whereas the only class IB member is termed p110γ.

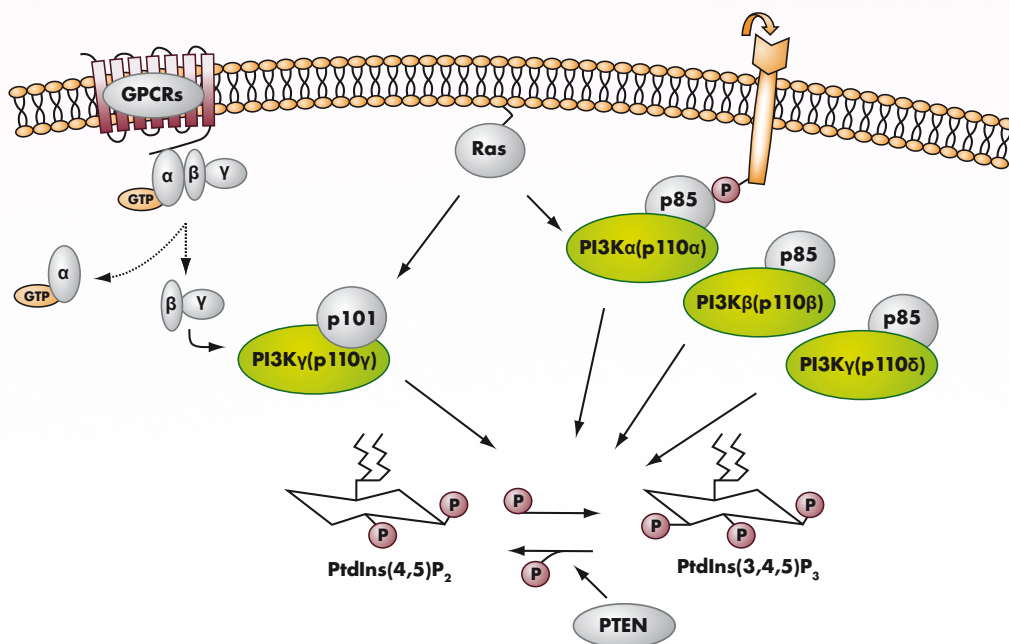
Class IA PI3Ks are commonly (but not exclusively) activated by tyrosine-kinases, which generate docking sites for the p85/p55 adaptor subunits by phosphorylating tyrosines within p85/p55 consensus binding motifs on a large number of proteins. For instance, class IA PI3Ks are targeted by antigen receptors, co-stimulatory and cytokine receptors.

In contrast, the class IB member p110γ is activated by G-protein-coupled receptors (GPCRs).

All class I PI3Ks are activated by the small GTPases Ras via a direct interaction between Ras-GTP and the catalytic p110 subunits.

Effectors of class I PI3Ks are pleckstrin-homology domain containing proteins such as Akt/PKB, BTK, TEC, ITK, BAM32, and small GTPases (e.g. Cdc42, Rac, or Ras).

The action of PI3Ks is antagonized by the phosphatidylinositol-3,4,5-trisphosphate phosphatases SHIP and PTEN.





## Alpha Isoform

### PI 3-Kinase alpha (PI3K $\alpha$ )

PI 3-Kinase alpha plays an important role in tumour progression and, particularly, in the control of proliferation, survival and regulation of the potential oncogene PKB. It has been further shown that the tumour suppressor PTEN is an antagonist of PI3K signalling and that somatic mutations of PI3K $\alpha$  are present in a variety of cancers. Some of the most frequent mutations in cancer constitutively activate PI3K $\alpha$  and may drive the oncogenic transformation

and chronic activation of downstream signalling commonly seen in cancer cells. Molecules important to the processes of metastasis, development of multi-drug resistance, angiogenesis and cell growth have been found to depend on PI3K $\alpha$  activity. PI3K $\alpha$  has been further validated as potential focus for cancer chemotherapy together with several additional PI3K effectors controlling cell proliferation and apoptosis.

PI 3-Kinases alpha	Accession No.	Cat.-No.	Amount	Price
<b>PI 3-Kinase alpha<sup>His</sup> (human)</b> Phosphoinositide 3-Kinase $\alpha^{\text{His}}$ , p110 $\alpha^{\text{His}}$ /p85 $\alpha$ human, recombinant, Sf9 cells	HSU79143 / NM_181523	PR-335	10 $\mu$ g	130 €
<b>PI 3-Kinase alpha (bovine*)</b> Phosphoinositide 3-Kinase $\alpha$ , p110 $\alpha$ /p85 $\alpha$ bovine, recombinant, Sf9 cells	NM_174574 / NM_174575	PR-341	10 $\mu$ g	130 €
<b>PI 3-Kinase alpha<sup>GST</sup> (bovine*)</b> Phosphoinositide 3-Kinase $\alpha^{\text{GST}}$ , p110 $\alpha^{\text{GST}}$ /p85 $\alpha$ bovine, recombinant, Sf9 cells	NM_174574 / NM_174575	PR-940	10 $\mu$ g	130 €

- Also available in 100  $\mu$ g and 1 mg size, please contact us for pricing and availability
- Several inactive mutants are available upon request.

\* Bovine PI 3-Kinase alpha differs from the human enzyme in only 2 positions, K532R and S535C.

#### References:

- Cantrell D.A. (2001) Phosphoinositide 3-kinase signalling pathways. *J. Cell Sci.* **114**:1439.  
 Foster et al. (2003) The phosphoinositide (PI) 3-kinase family. *J. Cell Science* **116**:3037.  
 Pirola et al. (2001) Activation Loop Sequences Confer Substrate Specificity to Phosphoinositide 3-Kinase  $\alpha$  (PI3K $\alpha$ ). *J. Biol. Chem.* **276**:21544.  
 Sayama et al. (2002) Phosphatidylinositol 3-kinase is a key regulator of early phase differentiation in keratinocytes. *J. Biol. Chem.* **277**:40390.  
 Stephens et al. (2005) Phosphoinositide 3-kinases as drug targets in cancer. *Pharmacology* **5**:357.



# PI 3-Kinase Family

## Beta Isoform

### PI 3-Kinase beta (PI3K $\beta$ )

PI3K $\beta$  can be activated by insulin via the insulin receptor to initiate a cascade of events that control cell growth and metabolism. PI3K $\beta$  activation is mediated by the interaction of the p85 regulatory subunit with tyrosine phosphorylated insulin receptor substrate (IRS) proteins (e.g. IRS-1 and IRS-2).

It was also shown that PI3K $\beta$  is involved in the control of apoptosis of human colon carcinoma cells. Furthermore, injection of neutralizing antibodies specific to PI3K $\beta$  in WiDr, HCT116 and CO 115 adenocarcinoma cells inhibited de novo DNA synthesis.

PI3K $\beta$  is the major PI3K isoform required for apoptotic cell and Fc- $\gamma$  receptor mediated phagocytosis shown for the Jurkat human leukemia T cell line and primary mouse macrophages.

Finally, beyond the canonical phosphotyrosine-dependent activation mechanisms, PI3K $\beta$  can be activated by G $\beta\gamma$  subunits of G-protein coupled receptors.

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PI 3-Kinases beta	Accession No.	Cat.-No.	Amount	Price
<b>PI 3-Kinase beta</b> Phosphoinositide 3-Kinase $\beta$ , p110 $\beta$ /p85 $\alpha$ human, recombinant, Sf9 cells	NM_006219 / NM_174575	PR-344	10 $\mu$ g	130 €

- Also available in 100  $\mu$ g and 1 mg size, please contact us for pricing and availability

#### References:

- Benistant et al. (2000) A specific function for phosphatidylinositol 3-kinase alpha (p85 $\alpha$ -p110 $\alpha$ ) in cell survival and for phosphatidylinositol 3-kinase beta (p85 $\alpha$ -p110 $\beta$ ) in de novo DNA synthesis of human colon carcinoma cells. *Oncogene* **19**:5083.
- Cantrell, D.A. (2001) Phosphoinositide 3-kinase signalling pathways. *J. Cell Sci.* **114**:1439.
- Foster et al. (2003) The phosphoinositide (PI) 3-kinase family. *J. Cell Sci.* **116**:3037.
- Leverrier et al. (2003) Class I phosphoinositide 3-kinase p110 $\beta$  is required for apoptotic cell and Fc $\gamma$  receptor-mediated phagocytosis by macrophages. *J. Biol. Chem.* **278**:38437.
- Murga et al. (2000) A Novel Role for Phosphatidylinositol 3-Kinase b in Signaling from G Protein-coupled Receptors to Akt. *J. Biol. Chem.* **275**:12069.
- Sayama et al. (2002) Phosphatidylinositol 3-kinase is a key regulator of early phasedifferentiation in keratinocytes. *J. Biol. Chem.* **277**:40390.
- Yart et al. (2002) A Function for Phosphoinositide 3-Kinase  $\beta$  Lipid Products in Coupling  $\beta\gamma$  to Ras Activation in Response to Lysophosphatidic Acid. *J. Biol. Chem.* **277**:21167.



## Gamma Isoform

### PI 3-Kinase gamma (PI3K $\gamma$ )

The only known class IB member PI 3-Kinase gamma exhibits limited tissue distribution. In contrast to the ubiquitously expressed p110 $\alpha$  and  $\beta$  enzymes, PI3K $\gamma$  is predominantly expressed in cells of hematopoietic origin. Neutrophil granulocytes from PI3K $\gamma$ -/- mice showed a reduced ability to respond to chemokines, and had a selective defect in the number of skin Langerhans cells and in lymph node CD8 $\alpha$ -DCs. Thus, plays a non-redundant role in dendritic cell (DC) trafficking and in the activation of specific immunity.

In macrophages, the chemokine RANTES/CCL5 activates the small GTPase Rac1 and its downstream target PAK2. This response depends on G $\beta$  activation and largely on the subsequent triggering of PI3K $\gamma$ .

PI 3-Kinases gamma	Accession No.	Cat.-No.	Amount	Price
<b>PI 3-Kinase gamma<sup>His</sup></b> Phosphoinositide 3-Kinase $\gamma^{\text{His}}$ , p110 $\gamma^{\text{His}}$ human, recombinant, Sf9 cells	NM_002649 / NP_002640	PR-343	10 $\mu$ g	130 €
<b>PI 3-Kinase gamma<sup>His</sup> / p101<sup>GST</sup></b> Phosphoinositide 3-Kinase $\gamma^{\text{His}}$ / p101 <sup>GST</sup> , p110 $\gamma^{\text{His}}$ /p101 <sup>GST</sup> human, recombinant, Sf9 cells	NM_002649 / NP_002640 / Y10742	PR-347	10 $\mu$ g	130 €

- Also available in 100  $\mu$ g and 1 mg size, please contact us for pricing and availability
- Several inactive mutants are available upon request.

#### References:

- Del Prete et al. (2004) Defective dendritic cell migration and activation of adaptive immunity in PI3Kgamma-deficient mice. *EMBO J.* **23**:3505.
- Foster et al. (2003) The phosphoinositide (PI) 3-kinase family. *J. Cell Science.* **116**:3037.
- Fuchikami et al. (2002) A Versatile High-Throughput Screen for Inhibitors of Lipid Kinase Activity: Development of an Immobilized Phospholipid Plate Assay for Phosphoinositide 3-kinase  $\gamma$ . *J. Biomol. Scr.* **7**:441.
- Kerchner et al. (2004) Differential Sensitivity of Phosphatidylinositol 3-Kinase p110 $\gamma$  to Isoforms of G Protein  $\beta$  Dimers. *J. Biol. Chem.* **279**:44554.
- Leopoldt et al. (1998) Gbetagamma stimulates phosphoinositide 3-kinase-gamma by direct interaction with two domains of the catalytic p110 subunit. *J. Biol. Chem.* **273**:7024.
- Weiss-Haljiti et al. (2004) Involvement of phosphoinositide 3-kinase gamma, Rac, and PAK signaling in chemokine-induced macrophage migration. *J. Biol. Chem.* **279**:43273.



# PI 3-Kinase Family

## Delta Isoform

### PI 3-Kinase delta (PI3K $\delta$ )

PI 3-Kinase delta is expressed primarily in blood-cell lineages, including cells that cause or mediate hematologic malignancies, inflammation, autoimmune diseases and allergies. Recently it was shown that the inactivation of PI3K $\delta$  in bone marrow mast cells (BBMCs) leads to defective stem cell factor-mediated proliferation, adhesion and migration of

these cells, and to impaired allergen-IgE-induced degranulation and cytokine release.

In neutrophils a role for PI3K $\delta$  in TNF $\alpha$ -induced signalling was demonstrated by a reduction in Akt-phosphorylation and PDK1 activity upon treatment with the  $\delta$ -specific inhibitor IC87114.

PI 3-Kinases delta	Accession No.	Cat.-No.	Amount	Price
<b>PI 3-Kinase delta</b> <sup>GST</sup> Phosphoinositide 3-Kinase $\delta$ <sup>GST</sup> , p110 $\delta$ <sup>GST</sup> /p85 $\alpha$ human, recombinant, Sf9 cells	O00329	PR-345	10 $\mu$ g	180 €

- Also available in 100  $\mu$ g and 1 mg size, please contact us for pricing and availability

#### References:

- Ali et al. (2004) Essential role for the p110 $\delta$  phosphoinositide 3-kinase in the allergic response. *Nature* **431**:1007.  
Foster et al. (2003) The phosphoinositide (PI) 3-kinase family. *J. Cell Science* **116**:3037.  
Puri et al. (2004) Mechanisms and implications of phosphoinositide 3-kinase  $\delta$  in promoting neutrophil trafficking into inflamed tissue. *Blood* **103**:3448.  
Sadhu et al. (2003) Essential role of phosphoinositide 3-kinase delta in neutrophil directional movement. *J Immunol.* **70**:2647.  
Sawyer et al. (2003) Regulation of breast cancer cell chemotaxis by the phosphoinositide 3-kinase p110delta. *Cancer Res.* **63**:1667.  
Vanhaesebroeck et al. (1999) Autophosphorylation of p110 $\delta$  phosphoinositide 3-kinase: a new paradigm for the regulation of lipid kinases in vitro and in vivo. *EMBO J.* **18**:1292.  
Zhang et al. (2002) Human platelets contain p110delta phosphoinositide 3-kinase. *Biochem Biophys Res Commun.* **296**:178.

## Adaptor Proteins

Activated PI 3-Kinases are composed of a catalytic subunit (p110alpha, p110beta or p110delta) associated with one of a large family of regulatory subunits (p85alpha, p85beta, p55gamma, p55alpha, and p50alpha). The p85 subunit exists in two isoforms p85alpha and p85beta. It contains two SH2 domains that bind to tyrosine-phosphorylated growth factor receptors or substrate adaptor proteins. It also contains a BH (breakpoint cluster region homology) domain that shows GAP activity towards the small GTPases Rab4, Rab5, Cdc42, Rac1 and

to a lesser extent towards Rab6 and Rab11. It was shown that p110alpha catalytic subunit mediated phosphorylation of the p85alpha adaptor reduces the lipid kinase activity of the heterodimer and this gives hints for PI3K-dependent signaling events not requiring production of 3'-phosphorylated phosphoinositides. p110alpha protein kinase activity is implicated in IRS 1 serine phosphorylation in insulin-treated adipocytes and in STAT3 and IRS 1 phosphorylation upon activation of the type 1 IFN receptor by IFN  $\alpha$ .



Adaptor Proteins	Accession No.	Cat.-No.	Amount	Price
<b>p85 alpha (bovine)</b> Phosphoinositide 3-Kinase, regulatory subunit bovine, recombinant, Leishmania tarentolae	NM_174575	PR-342	20 µg	190 €
<b>p85 beta (bovine)</b> Phosphoinositide 3-Kinase, regulatory subunit bovine, recombinant, Leishmania tarentolae	NP_777001.1	PR-942	10 µg	190 €

#### References:

- Cantrell, D.A. (2001) Phosphoinositide 3-kinase signalling pathways. *J. Cell Sci.* **114**:1439.
- Chamberlain et al. (2004) The p85α Subunit of Phosphatidylinositol 3'-Kinase Binds to and Stimulates the GTPase Activity of Rab Proteins. *J. Biol. Chem.* **279**:48607.
- Foster et al. (2003) The phosphoinositide (PI) 3-kinase family. *J. Cell Science* **116**:3037.
- Sayama et al. (2002) Phosphatidylinositol 3-kinase is a key regulator of early phasedifferentiation in keratinocytes. *J. Biol. Chem.* **277**:40390.
- Pirola et al. (2001) Activation Loop Sequences Confer Substrate Specificity to Phosphoinositide 3-Kinase α (PI3Kα). *J. Biol. Chem.* **276**:21544.

## Substrates

Phosphoinositide lipids serve as substrates for phosphoinositide 3-kinases (PI3Ks) that are capable of phosphorylating their 3' hydroxyl group. This process of generating lipid second messengers

regulates numerous biological processes including cell growth, differentiation, survival, proliferation, migration and metabolism.

Substrates	Cat.-No.	Amount	Price
<b>PI3K Lipid Substrate Mix 1</b> PI, PE, PS, PC, and SM	LI-011	1 mg (based on PI)	190 €
<b>PI3K Lipid Substrate Mix 2</b> PIP2, PE, PS, PC, and SM	LI-012	100 µg (based on PIP2)	190 €
<b>PI</b> L-α-Phosphatidylinositol, bovine liver	LI-001	5 mg	75 €
<b>PI</b> L-α-Phosphatidylinositol, synthetic	LI-008	100 µg	125 €
<b>PI-4-P</b> L-α-Phosphatidylinositol-4-phosphate, porcine brain	LI-002	1 mg	140 €
<b>PI-4-P</b> L-α-Phosphatidylinositol-4-phosphate, synthetic	LI-009	100 µg	125 €
<b>PI-4,5-P<sub>2</sub></b> L-α-Phosphatidylinositol-4,5-bisphosphate, porcine brain	LI-003	500 µg	140 €
<b>PI-4,5-P<sub>2</sub></b> L-α-Phosphatidylinositol-4,5-bisphosphate, synthetic	LI-010	100 µg	125 €



# PI 3-Kinase Family

Substrates	Cat.-No.	Amount	Price
<b>PC</b> L- $\alpha$ -Phosphatidylcholine, chicken egg	LI-004	5 mg	30 €
<b>PE</b> L- $\alpha$ -Phosphatidylethanolamine, chicken egg	LI-005	5 mg	30 €
<b>PS</b> L- $\alpha$ -Phosphatidylserine, porcine brain	LI-006	5 mg	30 €
<b>SM</b> Sphingomyelin, porcine brain	LI-007	5 mg	30 €

## References:

- Balla T. (2001) Pharmacology of phosphoinositides, regulators of multiple cellular functions. *Curr. Pharm. Des.* **7**:475.  
Foukas et al. (2002) Direct effects of caffeine and theophylline on p110 delta and other phosphoinositide 3-kinases. Differential effects on lipid kinase and protein kinase activities. *J. Biol. Chem.* **277**:37124.  
Martelli et al. (2004) Metabolism and signaling activities of nuclear lipids. *Cell Mol. Life Sci.* **61**:1143.  
Mozzi et al. (2003) Metabolism and functions of phosphatidylserine in mammalian brain. *Neurochem Res.* **28**:195.  
Wymann M.P. (2003) Phosphoinositide 3-kinase signalling – which way to target? *Trend Pharmacol. Sci.* **24**:323.  
Vanhaesebroeck et al. (2001) Synthesis and function of 3-phosphorylated inositol lipids. *Ann. Rev. Biochem.* **70**:535.

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## Inhibitors

Wortmannin is a cell-permeable, fungal metabolite that acts as a potent, selective and irreversible inhibitor of phosphatidylinositol 3-kinase (PI3K). It is active with purified preparations, cytosolic fractions and suitable for use with intact cells.

Wortmannin blocks the catalytic activity of PI3-Kinase with an IC<sub>50</sub> of 5 nM and without affecting upstream signalling events. It also inhibits the activities of myosin light chain kinase (MLCK), PI4-Kinase and phospholipase C (PLC) but at concentrations about 100 fold higher than those required for PI3K inhibition.

	Cat.-No.	Amount	Price
<b>Wortmannin</b> KY 12420	INH-001	1 mg	65 €

LY294002 is a cell-permeable compound that acts as a potent and selective inhibitor of phosphatidylinositol 3-kinase (PI3K). It is active with purified preparations, cytosolic fractions and suitable for use with intact cells.

LY294002 blocks the catalytic activity of PI3-Kinase with an IC<sub>50</sub> of 1.4  $\mu$ M and without affecting other kinases including PKC, PKA, MAPK, S6 kinase, EGFR, Src, or PI4-Kinase.

For intact cells like neutrophils, a concentration of 50  $\mu$ M completely abolishes the PI3K activity. It also inhibits the activities of myosin light chain kinase (MLCK), PI4-Kinase and phospholipase C (PLC) but at concentrations about 100 fold higher than those required for PI3K inhibition.

	Cat.-No.	Amount	Price
<b>LY294002</b>	INH-002	1 mg	35 €



Quercetin is a flavonoid with anticancer activity. It inhibits mitochondrial ATPase, phosphodiesterase, PI3-kinase activity ( $IC_{50}=3.8 \mu M$ ) and slightly PIP kinase activity. It has antiproliferative effects on cancer cell lines; reduces cancer cell growth via type II estrogen receptors and has been reported to arrest human leukemic T cells in late G1 phase of the cell cycle. It induces apoptosis in K562, Molt-4, Raji, A549 and MCAS tumor cell lines.

	Cat.-No.	Amount	Price
<b>Quercetin</b>	INH-003	100 mg	20 €

Myricetin is a flavonoid that differs from quercetin only by the addition of a hydroxyl at the 5'-OH of the phenyl moiety. It shows cytotoxic activity against several human leukemic cell lines in vitro. It strongly inhibits yeast  $\alpha$ -glucosidase, glyoxalase I in vitro, cow's milk xanthine oxidase, and PI3-Kinase ( $IC_{50}=1.8 \mu M$ ). Myricetin both modulates  $Na^+/K^+$ -ATPase-induced vasodilatation acting as a functional inhibitor of  $Na^+/K^+$ -ATPase activity and activates protein kinases, including PKC, to induce contraction. These effects appear to be related to the activation of PGH2-TXA2 receptors on vascular smooth muscle by the TXA2 released from endothelium.

	Cat.-No.	Amount	Price
<b>Myricetin</b>	INH-004	5 mg	18 €

Staurosporine is a potent cell permeable inhibitor of many kinases including Protein Kinase C, Protein Kinase A, CaM kinase, myosin light chain kinase, and PI3K ( $IC_{50}=9 \mu M$ ).

	Cat.-No.	Amount	Price
<b>Staurosporine</b> Antibiotic AM 2282	INH-005	50 $\mu g$	65 €

The biological effects of Staurosporine include cytotoxicity, relaxation of smooth muscle, and regulation of eNOS gene expression. It inhibits platelet aggregation induced by collagen or ADP but has no effect on thrombin-induced platelet aggregation. Staurosporine induces apoptosis in human malignant glioma cell lines and arrests normal cells at the G1 checkpoint.

**References:**

Dimas et al. (2000) Biological activity of myricetin and its derivatives against human leukemic cell lines in vitro. *Pharmacol Res.* **42**:475.

Jimenez et al. (2002) Involvement of protein kinase C and  $Na^+/K^+$ -ATPase in the contractile response induced by myricetin in rat isolated aorta. *Planta Med.* **68**:133.

Lee S.K. and Stern P.H. (2000) Divergent effects of protein kinase C (PKC) inhibitors staurosporine and bisindolylmaleimide I (GF109203X) on bone resorption. *Biochem. Pharmacol.* **60**:923.

Semba et al. (2002) The in vitro and in vivo effects of 2-(4-morpholinyl)-8-phenyl-chromone (LY294002), a specific inhibitor of phosphatidylinositol 3'-kinase, in human colon cancer cells. *Clin. Cancer Res.* **8**:1957.

Walker et al. (2000) Structural Determinants of Phosphoinositide 3-Kinase Inhibition by Wortmannin, LY294002, Quercetin, Myricetin, and Staurosporine. *Mol. Cell.* **6**:909.

Wan et al. (2002) PTEN augments staurosporine-induced apoptosis in PTEN-null Ishikawa cells by downregulating PI3K/Akt signalling pathway. *Cell Death Differ.* **9**:414.



# PI 3-Kinase Family

## PI 3-Kinase Antibodies

Anti-PI3 Kinase gamma <i>mouse monoclonal antibody</i>	Cat.-No.	Amount	Price
cell culture supernatant recognizes a sequence between aa 97-335	ABD-026S	200 µl	70 €
	ABD-026L	1 ml	280 €
cell culture supernatant	ABD-027	1 ml	200 €
purified	ABD-027P	100 µg	350 €

### References:

Leopoldt D. et al. (1998) Gbetagamma stimulates phosphoinositide 3-kinase-gamma by direct interaction with two domains of the catalytic p110 subunit. *J. Biol. Chem.* 273:7024.

## PI 3-Kinase Assay

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Sensitive assay for detection of PI 3-Kinase activity and screening of inhibitors.

PI 3-Kinase Assay Kit	Cat.-No.	Amount	Price
2 × Reaction Buffer, Lipid Substrate Mix, ATP and Control PI3K	PR-943	8 × 12 assay points	350 €

### References:

Ahn et al. (2004) PIKE (Phosphatidylinositol 3-Kinase Enhancer)-A GTPase Stimulates Akt Activity and Mediates Cellular Invasion. *J. Biol. Chem.* **279-16**:16441.

Dan et al. (2004) Phosphatidylinositol-3-OH kinase/AKT and survivin pathways as critical targets for geranylgeranyltransferase I inhibitor-induced apoptosis. *Oncogene* **23**:706.

Fruman et al. (1998) Phosphoinositide kinases. *Annu Rev Biochem.* **67**:481-507.



## PI 3-Kinase Assay Protocol

- 2 × conc. Reaction Buffer
  - 20 mM TrisHCl pH 7.4
  - 2 mM MgCl<sub>2</sub>
  - 50 mM NaCl
- ATP Mix
  - 240 μM ATP
  - 1 μCi γ<sup>32</sup>P-ATP / 10 μl
- Lipid Substrate Vesicles
  - Prepare 800 μM phosphatidylinositol in water
  - Sonicate the emulsion 1 h in a water bath sonicator



### Kinase Assay

- Prepare a premix of the following components for each assay point.
  - 25 μl Reaction Solution
  - 10 μl Lipid Substrate Vesicles
  - 5 μl PI3K Sample (dilute as appropriate)
- Incubate 5 min at room temperature
- Add 10 μl ATP Mix to start the reaction
- Incubate 15 min at 37°C
- Stop reaction by addition of 100 μl 1 M HCl and vortexing

### Extraction of the lipids

- Add 300 μl of chloroform/methanol (1:1)
- Vortex and centrifuge to separate the phases
- Remove the upper aqueous phase
- Wash the organic layer with 200 μl 1 M HCl and remove the water phase
- Transfer 100 μl of the organic phase onto a silica gel TLC plate and run in 2 M acetic acid/isopropanol (1:2)
- Expose the plate for visualization and quantification of phosphatidylinositol 3-phosphate spots
- Alternatively, an aliquot of the washed chloroform phase may be counted by liquid scintillation



# Terms and Conditions of Sales

## Ordering

The following options are available for ordering products directly from Jena Bioscience:

- Mail orders
- Telephone orders
- 24 hour fax ordering
- Online ordering

Please provide the following information when ordering:

- Your name, name of institution
- Billing and shipping address
- PO number (if applicable)
- Catalog number of products and quantity needed
- Contact person and contact data for questions

### Mail orders

Please send your mail orders to the following address:

Jena Bioscience GmbH  
Loebstedter Strasse 80  
07749 Jena, Germany

### Telephone orders

We will accept telephone orders from Monday to Friday between 8 am and 4 pm Central European Time.  
+49 – 3641 – 628 5000

### 24 hour fax ordering

Please send your fax order to:  
+49 – 3641 – 628 5100

### Online ordering

Jena Bioscience products can be ordered online. When ordering by e-mail, please direct your orders to: [orders@jenabioscience.com](mailto:orders@jenabioscience.com)

Products can also be ordered online through our online shop. Go to <http://www.jenabioscience.com> and follow the instructions.

### Important Notice:

*Products that have been ordered by mistake cannot be returned to Jena Bioscience. Products that are returned unrequestedly to Jena Bioscience will not be accepted, but fully charged to the customer's account.*

### Shipping

All customers will receive a fax confirmation of the order with invoice and shipping waybill number. Products that do not require cooling as well as products that can be shipped on blue ice are shipped by UPS Express service. Proteins and other products that need to be sent on dry ice are shipped by FedEx. Domestic shipments within Germany are sent by General Overnight Express service.

If you wish your order to be shipped by a different carrier, please contact us and provide all necessary information with your order.

All orders are shipped FCA/FOB (Incoterms 2000).

### Prices and Charges

Please note that the prices of products in the catalog and on our website do not include freight charges, duties, taxes or customs fees.

Freight charges will be prepaid and added to the invoice. Freight charges for online orders are indicated when you check out of the online store. If you need information on freight charges for your particular order, please contact us with all necessary information.

Jena Bioscience will not pay any duties, taxes or customs fees.

Products and prices are subject to change without notice. Current pricing will be confirmed at the time of your order. No minimum order required.

## Payment

Invoices will be issued after your order has been shipped and will be sent to the billing address by separate mail. Invoices will not be included within the shipments. In case of partial deliveries, separate invoices will be issued after each shipment has left Jena Bioscience. You will find payment information (bank addresses and account data) on each invoice. Jena Bioscience accepts payment by:

### Check

Please send your payment checks to the following address:

Jena Bioscience GmbH  
Loebstedter Strasse 80  
07749 Jena, Germany

We kindly ask you to make sure that our invoice number and your customer number appear on the cheque.

### Wire transfer

Please remit your payments to one of the following bank accounts:

Bayerische Hypo- und Vereinsbank AG	Account No.: 4196090
Niederlassung Thüringen	Bank code (BLZ): 83020087
Schillerstrasse 4	IBAN: DE 05830200870004196090
07745 Jena, Germany	SWIFT: HYVEDEMM463

Sparkasse Jena-Saale-Holzland	Account No.: 32417
Ludwig-Weimar-Gasse 5	Bank code (BLZ): 83053030
07743 Jena, Germany	IBAN: DE 22830530300000032417

### Credit card

Jena Bioscience accepts the following credit cards:

- VISA
- Mastercard
- American Express



If you wish to pay by credit card, please provide the following credit card information:

- Card holder
- Card number
- Expiry date
- Security code (VISA / Mastercard: 3 digits, to be found on your card's back side in the upper right corner of the signature field; AmEx: usually 4 digits (sometimes only three), to be found on the front side of your card above the card number)

### Patent Disclaimer

Unless explicitly stated, no license or immunity under any patent is either granted or implied by the sale of any of our products. Jena Bioscience does not warrant that the resale or use of its products delivered will not infringe the claims of any patent, trademark or copyright covering the use of the product itself or its use in the operation of any process. Furthermore, the purchaser assumes all risks of patent, trademark or copyright infringement associated with any such use, combination or operation.

# Jena Bioscience Fax Order Form

Please copy this page, fill in your order and fax it to: **+49-3641-628-5100**

## Shipping address

## Billing address

Name	Customer number
University/Company	University/Company
Institute/Department	Institute/Department
Address	Address
Postcode	Postcode
City/Country	City/Country
Phone	VAT number (EEC only)
Fax	PO number
Email	Date/Signature

If you wish to pay by credit card, please provide the following credit card information:

I want to pay by


Card holder  Card number

Expiry date  Security code

(VISA / Mastercard: 3 digits on card's back side, upper right corner of signature field; AmEx: 4 digits, card's front side, above card number)

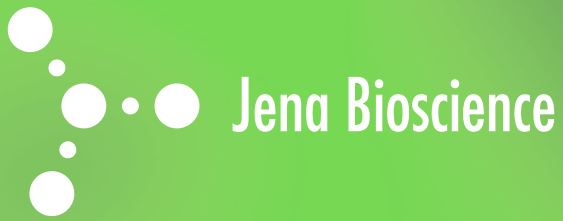
	Catalog number	Product	Quantity	Net Price per Item EURO	Net Price all Items EURO
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
				Total	



**Jena Bioscience**  
www.jenabioscience.com

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Loebstedter Str. 80  
07749 Jena  
Germany

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Fax +49 (0)3641-628-5100  
info@jenabioscience.com  
www.jenabioscience.com



## How to find us in Germany



For inquiries or further information,  
please contact: [info@jenabioscience.com](mailto:info@jenabioscience.com)

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07749 Jena, Germany  
Phone +49 (0)3641-628-5000  
Fax +49 (0)3641-628-5100

For the complete Jena Bioscience product portfolio please view  
[www.jenabioscience.com](http://www.jenabioscience.com)