# Anti-PI 3 Kinase p85 alpha Antibody R1312-6

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 84 kDa
Description:	Phosphatidylinositol 3-kinase regulatory subunit alpha is an enzyme that in humans is encoded by the PIK3R1 gene. Phosphatidylinositol 3-kinase phosphorylates the inositol ring of phosphatidylinositol at the 3-prime position. The enzyme comprises a 110 kD catalytic subunit and a regulatory subunit of either 85, 55, or 50 kD. This gene encodes the 85 kD regulatory subunit. Phosphatidylinositol 3-kinase plays an important role in the metabolic actions of insulin, and a mutation in this gene has been associated with insulin resistance. Alternative splicing of this gene results in three transcript variants encoding different isoforms. Mutations in PIK3R1 are implicated in cases of breast cancer.Mutations in PIK3R1 are associated to SHORT syndrome.
lmmunogen:	Synthetic peptide within C-terminal human PI3-kinase p85 subunit alpha.
Positive control:	Jurkat, PC12, NIH/3T3, HepG2, human liver tissue, human kidney tissue, mouse colon tissue, Hela.
Subcellular location:	Cytoplasm, nucleus
Database links:	SwissProt: P27986 Human   P26450 Mouse   Q63787 Rat
Recommended Dilutions: WB IF-Cell IHC	1:1,000 1:100-1:200 1:200-1:500
Storage Buffer:	1*PBS (pH7.4), 0.2% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4 $^\circ\!C$ after thawing. Aliquot store at -20 $^\circ\!C$ or -80 $^\circ\!C$ . Avoid repeated freeze / thaw cycles.
Purity:	Immunogen affinity purified.

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#### Images



**Fig1:** Western blot analysis on cell lysates using anti- PI3-kinase p85 subunit alpha rabbit polyclonal antibodies.



**Fig2:** ICC staining PI3-kinase p85 subunit alpha in Hela cells (green). Cells were fixed in paraformaldehyde, permeabilised with 0.25% Triton X100/PBS.



**Fig3:** Immunocytochemistry analysis of HepG2 cells labeling PI 3 Kinase p85 alpha with Rabbit anti-PI 3 Kinase p85 alpha antibody (R1312-6) at 1/200 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37  $^{\circ}$ C, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-PI 3 Kinase p85 alpha antibody (R1312-6) at 1/200 dilution in 2% negative goat serum overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor <sup>TM</sup> 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

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**Fig4:** Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-PI 3 Kinase p85 alpha antibody (R1312-6) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (R1312-6) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig5:** Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-PI 3 Kinase p85 alpha antibody (R1312-6) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (R1312-6) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.



**Fig6:** Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-PI 3 Kinase p85 alpha antibody (R1312-6) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (R1312-6) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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**Fig7:** Flow cytometric analysis of Hela cells labeling PI 3 Kinase p85 alpha.

Cells were fixed and permeabilized. Then stained with the primary antibody (R1312-6, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor<sup>TM</sup> 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

#### Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

#### **Background References**

- "Natural variants of human p85 alpha phosphoinositide 3-kinase in severe insulin resistance: a novel variant with impaired insulin-stimulated lipid kinase activity." Baynes K.C.R., Beeton C.A., Panayotou G., Stein R., Soos M., Hansen T., Simpson H., O'Rahilly S., Shepherd P.R., Whitehead J.P. Diabetologia 43:321-331(2000)
- "The structure of a human p110alpha/p85alpha complex elucidates the effects of oncogenic PI3Kalpha mutations." Huang C.-H., Mandelker D., Schmidt-Kittler O., Samuels Y., Velculescu V.E., Kinzler K.W., Vogelstein B., Gabelli S.B., Amzel L.M. Science 318:1744-1748(2007)

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