

# PI3 Kinase Antibody Sampler Kit



✓ 1 Kit  
(6 x 40 µl)

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-PI3K p85 (Tyr458)/p55 (Tyr199) Antibody	4228	40 µl	60, 85 kDa	Rabbit IgG
PI3 Kinase p85 (19H8) Rabbit mAb	4257	40 µl	85 kDa	Rabbit IgG
PI3 Kinase p110α (C73F8) Rabbit mAb	4249	40 µl	110 kDa	Rabbit IgG
PI3 Kinase p110β (C33D4) Rabbit mAb	3011	40 µl	110 kDa	Rabbit IgG
PI3 Kinase p110-γ (D55D5) Rabbit mAb	5405	40 µl	110 kDa	Rabbit IgG
PI3 Kinase Class III (D4E2) Rabbit mAb	3358	40 µl	100 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions and additional application protocols.

**Description:** The PI3 Kinase Sampler Kit provides an economical means of studying PI3 kinase subunits in cells. The kit contains enough primary and secondary antibodies to perform four Western blot experiments per primary antibody.

**Background:** Phosphoinositide 3-kinase (PI3K) catalyzes the production of phosphatidylinositol-3,4,5-triphosphate by phosphorylating phosphatidylinositol (PI), phosphatidylinositol-4-phosphate (PIP) and phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>). Growth factors and hormones trigger this phosphorylation event, which in turn coordinates cell growth, cell cycle entry, cell migration, and cell survival (1). PTEN reverses this process, and the PI3K signaling pathway is constitutively activated in human cancers that have loss of function of PTEN (2). PI3Ks are composed of a catalytic subunit (p110) and a regulatory subunit. Various isoforms of the catalytic subunit (p110α, p110β, p110γ, and p110δ) have been isolated, and the regulatory subunits that associate with p110α, p110β, and p110δ are p85α and p85β (3). In contrast, p110γ associates with a p101 regulatory subunit that is unrelated to p85. Furthermore, p110γ is activated by βγ subunits of heterotrimeric G proteins (4).

**Specificity/Sensitivity:** Each antibody in the PI3 Kinase Antibody Sampler Kit recognizes only its specific target and does not cross-react with other family members. Each total antibody detects endogenous levels of its target protein. Phospho-PI3K p85 (Tyr458)/p55 (Tyr199) Antibody detects endogenous levels of p85/p55 only when phosphorylated at Tyr458/Tyr199.

**Source/Purification:** Phospho-specific polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr458 of mouse p85. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to the sequence of human PI3K p85, corresponding to human PI3K p110-gamma, surrounding Asp520 from human PI3K p110α, containing human PI3K p110β, and the amino-terminal sequence of human PI3K class III.

**Background References:**

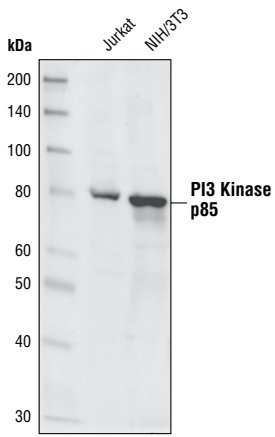
- (1) Cantley, L.C. (2002) *Science* 296, 1655-7.
- (2) Simpson, L. and Parsons, R. (2001) *Exp Cell Res* 264, 29-41.
- (3) Neri, L.M. et al. (2002) *Biochim Biophys Acta* 1584, 73-80.
- (4) Stoyanov, B. et al. (1995) *Science* 269, 690-3.

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibodies.

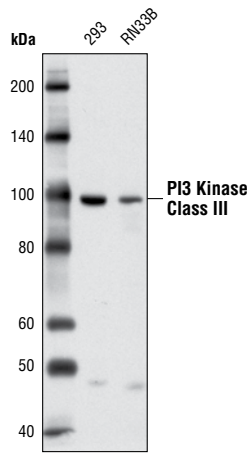
**Recommended Antibody Dilutions:**  
Western blotting 1:1000

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

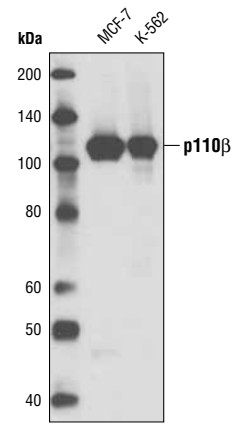
Selected rabbit monoclonal antibodies are produced under license (granting certain rights including those under U. S. Patent No. 5,675,063 and/or U.S.S.N. 11/476,277) from Epitomics, Inc. U.S.S.N. 11/476,277) from Epitomics, Inc.



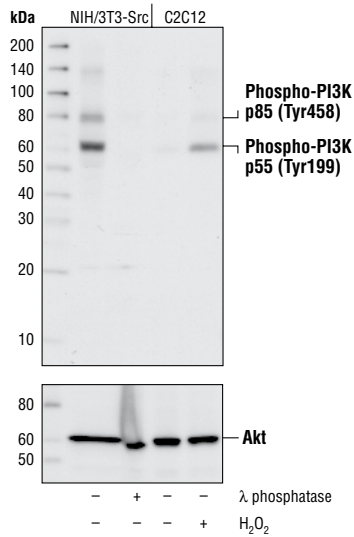
Western blot analysis of extracts from Jurkat and NIH/3T3 cells using **PI3 Kinase p85 (19H8) Rabbit mAb #4257**.



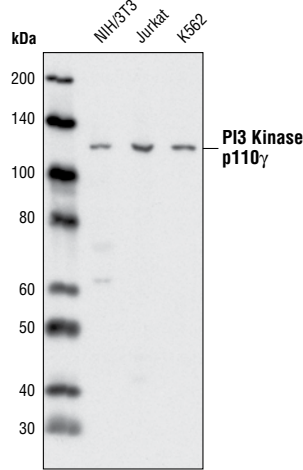
Western blot analysis of extracts from 293 and RN33B cells using **PI3 Kinase Class III (D4E2) Rabbit mAb #3358**.



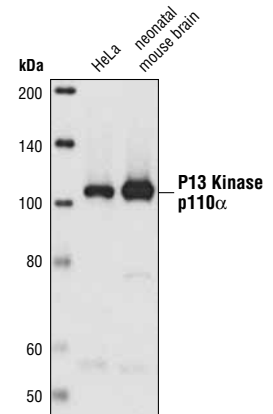
Western blot analysis of extracts from MCF-7 and K-562 cells using **PI3 Kinase p110β (C33D4) Rabbit mAb #3011**.



Western blot analysis of extracts from NIH/3T3-Src cells, untreated or treated with  $\lambda$  phosphatase, and from C2C12 cells, untreated or treated with  $H_2O_2$ , using **Phospho-PI3K p85 (Tyr458)/p55 (Tyr199) Antibody #4228**.



Western blot analysis of extracts from NIH/3T3, Jurkat and K562 cells using **PI3 Kinase p110-γ (D55D5) Rabbit mAb**.



Western blot analysis of extracts from HeLa cells and neonatal mouse brain using **PI3 Kinase p110α (C73F8) Rabbit mAb #4249**.

## Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO<sup>®</sup> incubation and declines over the following 2 hours.