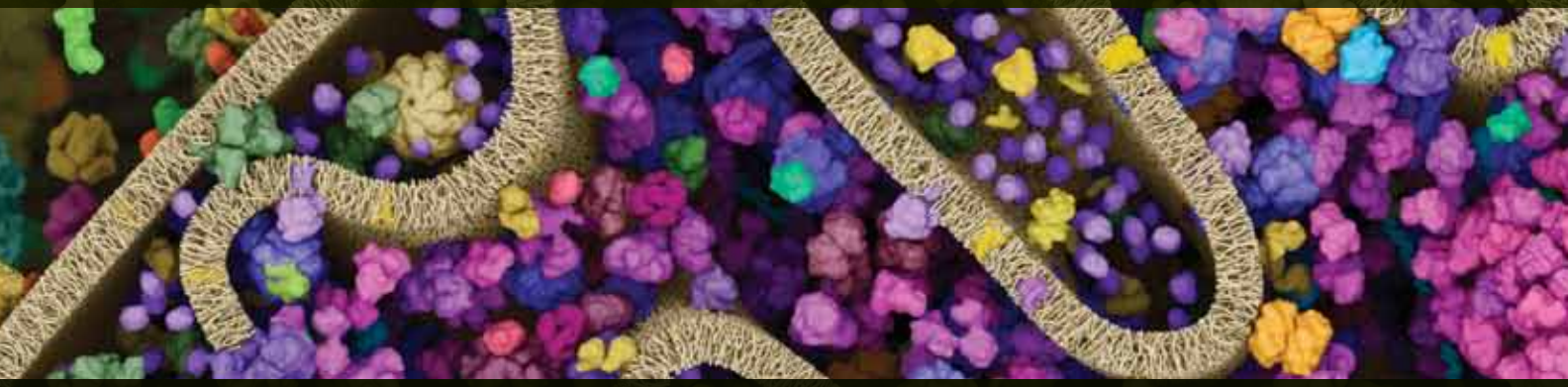


ANTIBODIES, KITS, AND REAGENTS FOR THE STUDY OF

Cellular Metabolism

Cell Signaling TECHNOLOGY®

Cellular Metabolism

Organisms continually process nutrients for energy production and creation of new biomass. The study of how and when substrates are metabolized is central to the study of cell proliferation, growth, survival, and tumor progression.

Glucose is the primary energy source for most cells of the body, and the maintenance of glucose homeostasis is an essential physiological process regulated by hormones. An elevation in blood glucose levels during feeding stimulates insulin release from pancreatic β-cells. Insulin is a hormone that regulates glucose and lipid metabolism throughout the body. It stimulates glucose uptake in muscle and adipose tissue, increases glycogen synthesis and suppresses gluconeogenesis in the liver, and promotes triglyceride synthesis and suppression of lipolysis in adipose tissue. Insulin binding to the insulin receptor (IR) triggers signaling through Akt to cause translocation of Glut4-containing vesicles from intracellular compartments to the cell surface, thereby allowing glucose to enter the cell resulting in decreased blood glucose concentrations. In addition, IR-mediated activation of Akt and MAPK results in cell growth, protein synthesis, and survival. An ineffective response to insulin produces the insulin resistance associated with type 2 diabetes (T2D), metabolic syndrome, and some forms of obesity.

Once glucose enters the cell, it is metabolized via glycolysis and the Krebs cycle for optimal ATP production. However, this metabolic signature is disrupted in some cancer cells, which display a shift towards ATP production via glycolysis alone. This phenomenon is called aerobic glycolysis or the Warburg effect. Signaling proteins such as Akt, AMPK, MAPK, p53, and c-Myc influence aerobic glycolysis by regulating expression and activity of key metabolic enzymes.

As blood glucose concentrations fall, the hormone glucagon is released from pancreatic α-cells. Glucagon has the opposite effect of insulin—it promotes glycogenolysis and gluconeogenesis in the liver in order to sustain optimal blood glucose levels. In addition to the actions of glucagon, the serine/threonine kinase AMPK also plays an important role in maintaining cellular energy levels. AMPK is activated by increases in the AMP:ATP and/or ADP:ATP ratios, as a result of low ATP levels. AMPK functions to restore cellular energy levels by promoting ATP-generating processes such as glucose transport, fatty acid oxidation, and glycolysis, while inhibiting ATP-consuming processes such as protein and lipid synthesis. AMPK's role as a master metabolic regulator has made it the focus of intense research efforts within the fields of cancer and metabolic and cardiovascular diseases. For example, the AMPK activator, metformin, is one of the most commonly prescribed drugs for the treatment of T2D.

Select Reviews: Al-Goblan, A.S., Al-Alfi, M.A., and Khan, M.Z. (2014) *Diabetes Metab. Syndr. Obes.* 7, 587–591. Du, Y. and Wei, T. (2014) *Protein Cell* 5, 203–213. Elf, S.E. and Chen, J. (2014) *Cancer* 120, 774–780. Hardie, D.G. (2014) *Curr. Opin. Cell Biol.* 33C, 1–7. Hers, I., Vincent, E.E., and Tavaré, J.M. (2011) *Cell Signaling* 23, 1515–1527.

Rachel, Research Associate has been with CST since 2008.

CST has antibodies, kits, and reagents

for each stage of the experimental process.

Primary Antibodies

Over 740 primary antibodies directed against more than 300 protein targets. The collection is continually expanding, so please check our website frequently for a complete, up-to-date product list.

PTMScan Kits and Services

PTMScan® Kits and Services utilize motif antibodies and LC-MS/MS technology to generate quantitative profiles of hundreds to thousands of proteins containing a particular type of post-translation modification.

Antibody Sampler Kits

Sampler kits allow for the simultaneous analysis of multiple nodes in a pathway of interest or modification sites within a protein of interest.

ELISA Kits and Antibody Arrays

PathScan® ELISA Kits enable you to scale up your analysis to a 96-well format (384-well plates are also available on a custom basis), while antibody arrays allow you to monitor multiple pathway nodes in parallel using sandwich assays in a slide- or membrane-based array.

SignalSilence siRNA

Rigorously validated SignalSilence® siRNAs can be used to selectively reduce the expression of a protein of interest.

Chromatin IP Kits and Reagents

SimpleChIP® and SimpleChIP® Plus Chromatin IP Kits, ChIP-validated antibodies, control PCR primers, and companion products needed to perform successful ChIP assays are available to explore protein-DNA interactions.

Experimental Controls

Control cell extracts, control proteins, blocking peptides, and isotype controls are available to help you verify antibody specificity.

Companion Products

Secondary antibodies, loading controls, buffers, dyes, chemical modulators, detection reagents, protease inhibitors, and peptide standards are available to support your protocol.

Custom Products

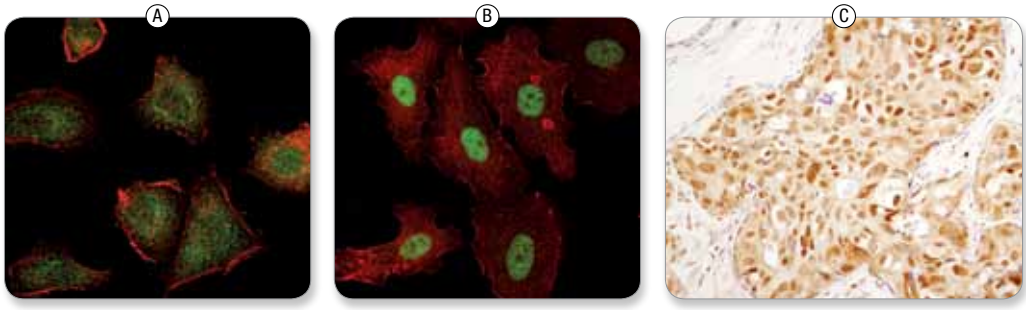
Our custom antibody department will work with you if you require a product in a specific size or formulation for your particular assay platform or if you need a product validated using a specific measure or assay.

Tools to move your research forward

TARGET LOCALIZATION

Antibodies, both conjugated and unconjugated, to assess subcellular localization of key metabolic targets

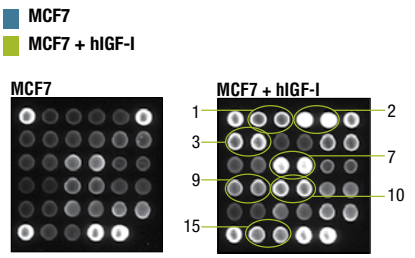
Fox03a (D19A7) Rabbit mAb #12829: Confocal IF analysis of PC-3 cells, treated with Human Insulin-like Growth Factor I (hIGF-I) #8917 **(A)** or LY294002 #9901 **(B)**, using #12829 (green). Actin filaments were labeled with DY-554 phalloidin (red). IHC analysis of paraffin-embedded human breast carcinoma **(C)** using #12829.

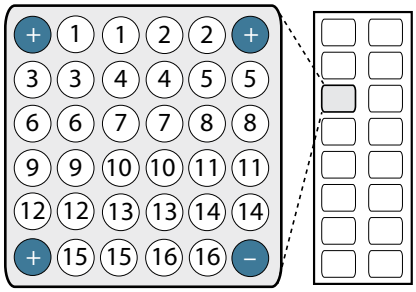


QUANTITATIVE ASSAYS

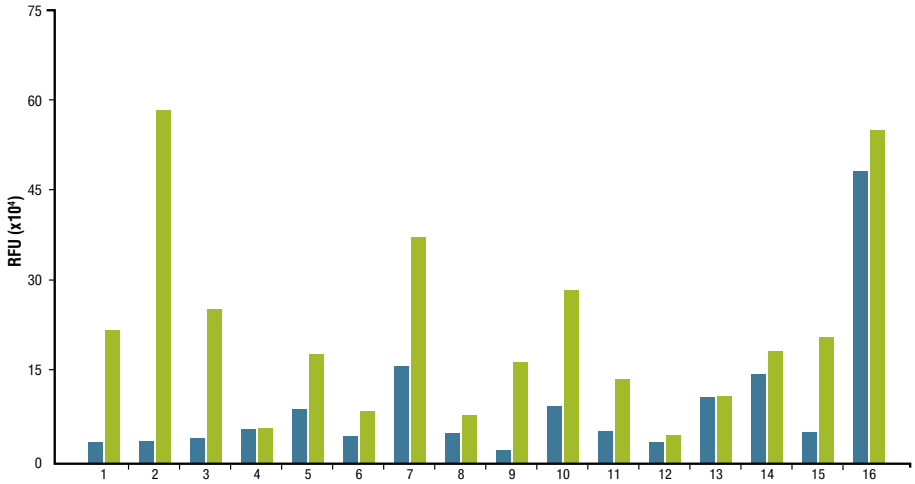
ELISA kits, antibody arrays, chromatin IP kits, and other cell-based assays for quantitative analysis

PathScan® Akt Signaling Antibody Array Kit (Fluorescent Readout) #9700: MCF7 cells were grown to 85% confluency and then serum starved overnight. Cells were either untreated or treated with Human Insulin-like Growth Factor I (hIGF-I) #8917 (100 ng/ml, 20 min). Cell extracts were prepared and analyzed with #9700. Images were acquired using the LI-COR® Biosciences Odyssey® imaging system. Graph shows quantification of results. Pixel intensity was quantified using the LI-COR® Image Studio v2.0 array analysis software.





Target Map: Using PathScan® Akt Signaling Antibody Array Kit (Fluorescent Readout) #9700.



Targets	Phosphorylation Site
+ Positive Control	N/A
− Negative Control	N/A
1. Akt	Thr308
2. Akt	Ser473
3. S6 Ribosomal Protein	Ser235/236
4. AMPKα	Thr172
5. PRAS40	Thr246
6. mTOR	Ser2481
7. GSK-3α	Ser21

Targets	Phosphorylation Site
8. GSK-3β	Ser9
9. p70 S6 Kinase	Thr389
10. p70 S6 Kinase	Thr421/Ser424
11. Bad	Ser112
12. RSK1	Ser380
13. PTEN	Ser380
14. PDK1	Ser241
15. Erk1/2	Thr202/Tyr204
16. 4E-BP1	Thr37/46

PathScan Antibody Array Kits

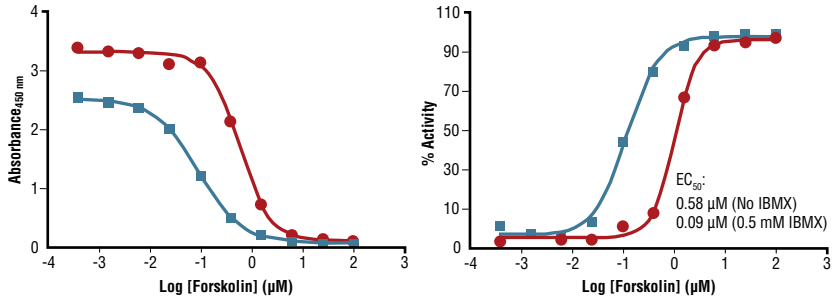
PathScan® Antibody Array Kits allow for analysis of a number of well-characterized proteins or protein modifications in a single experiment, saving you valuable reagents and time. Visit our website for a complete listing of PathScan Antibody Array Kits.

- **Multiplex Format:** Analyze numerous carefully selected signaling targets simultaneously
- **Multiple Array Pads:** Test up to 32 experimental variables in parallel generating up to 624 data points per kit for rich experimental design
- **Low Sample Volume:** Use as little as 50 µl of sample (0.2–0.5 mg/ml total protein) per pad

CELL-BASED ASSAYS

Second messenger, proliferation, viability, and cell death assays to monitor cell health and signaling in a cell population

Cyclic AMP XP® Assay Kit #4339: Treatment of CHO cells with Forskolin #3828 increases cAMP concentration as detected by #4339. CHO cells were seeded at $4x10^4$ cells/well in a 96-well plate and incubated overnight. Cells were either left untreated or pretreated with 0.5 mM IBMX for 30 min prior to forskolin treatment (15 min) and lysed with 1X Cell Lysis Buffer #9803. The absorbance values (left) and percentage of activity (right) are shown above. The percentage of activity is calculated as follows: % activity=100x[$(A-A_{basal})/(A_{max}-A_{basal})$], where A is the sample absorbance, A_{max} is the absorbance at maximum stimulation (i.e., high forskolin concentration), and A_{basal} is the absorbance at basal level (no forskolin). Forskolin directly activates adenylyl cyclases and increases cellular cAMP concentration. IBMX is a non-specific inhibitor of cAMP and cGMP phosphodiesterases and promotes accumulation of cAMP and cGMP in cells.

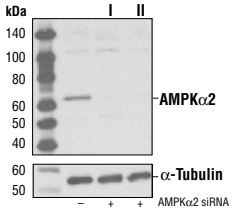


● **Insulin-treated**
■ **Untreated**

EXPERIMENTAL CONTROLS

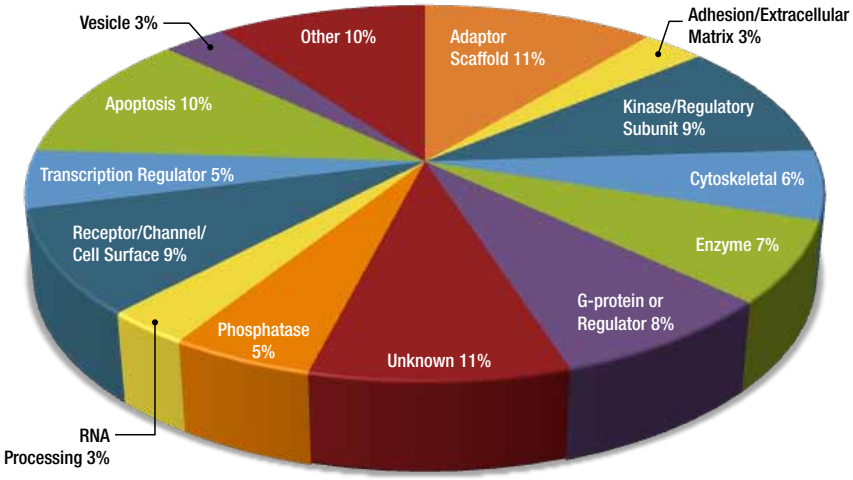
Alter protein expression with controlled siRNA knockdown

SignalSilence® AMPKα2 siRNA I #6620 and SignalSilence® AMPKα2 siRNA II #6630: WB analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), #6620(+), or #6630 (+), using AMPKα2 Antibody #2757 (upper) or α-Tubulin (11H10) Rabbit mAb #2125 (lower). The AMPKα2 Antibody confirms silencing of AMPKα2 expression, while the α-Tubulin (11H10) Rabbit mAb is used as a loading control.



PROTEOMIC ANALYSIS

PTMScan® Kits and Services for PTM profiling



PTMScan® Phospho-AMPK Substrate Motif (LXRXXS*/T*) Kit #5564: Chart showing the relative category distribution of proteins with phosphorylated residues identified from peptides from mouse liver tissue using #5564.

PTMScan Technology

PTMScan® Technology is a proprietary proteomic method developed at Cell Signaling Technology designed to help researchers understand the role of post-translational modifications (PTMs) in both health and disease. This technology can be used to assess PTMs associated with a disease model system, detect substrates of novel signaling proteins, profile effects of a candidate therapeutic on a specific type of PTM, or identify novel biomarkers to predict therapeutic efficacy.

Learn more at www.cellsignal.com/proteomics

Cellular Metabolism Targets

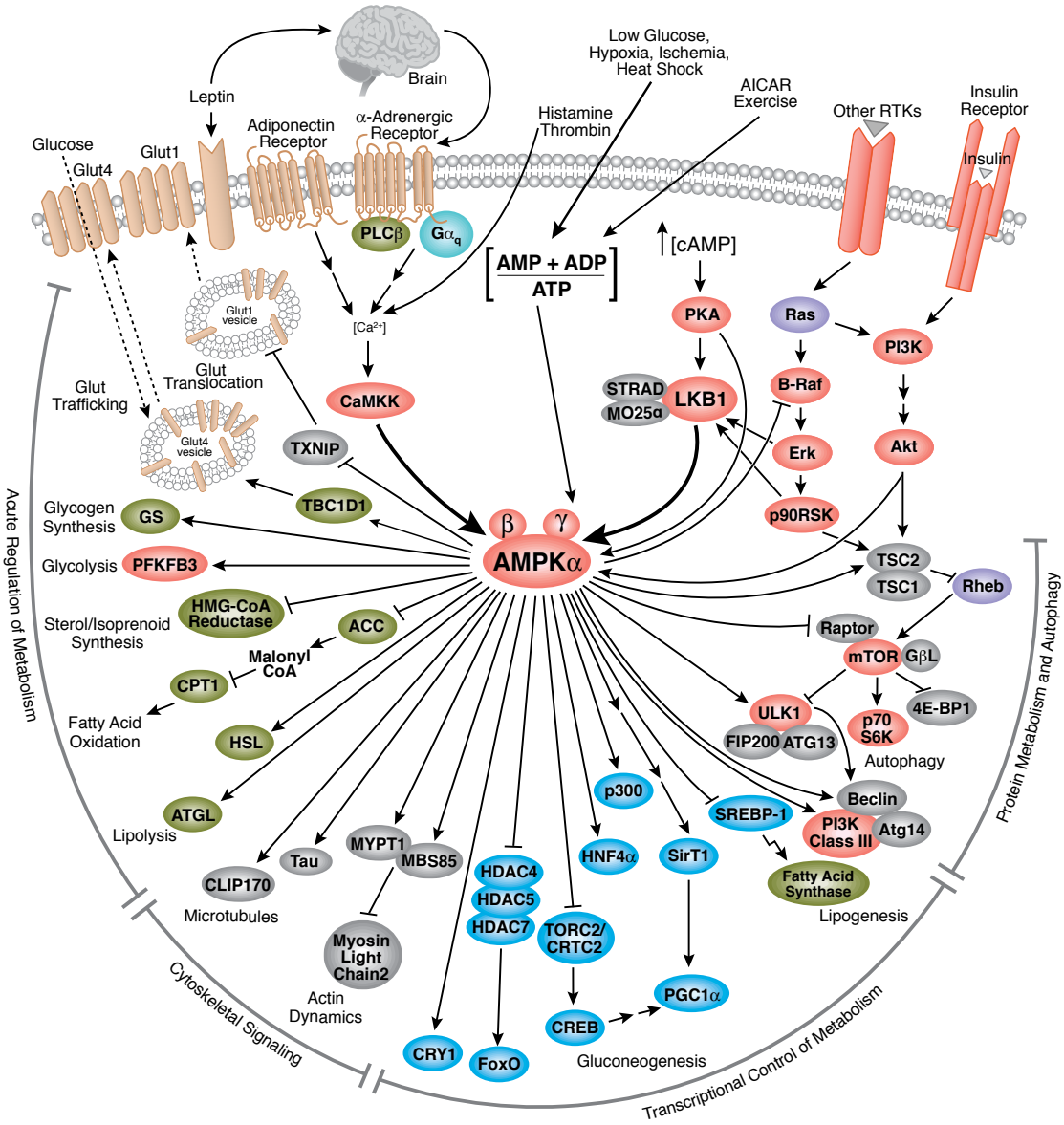
WE'VE GOT IT COVERED

Our total and modification-specific antibody portfolio covers critical targets within metabolic pathways. Visit our website for a complete product listing.

- ABCB1
- ABCC1
- ABCC3
- ABCC4
- ABCG2
- ACAD9
- ACAT2
- AceCS1
- Acetyl-CoA Carboxylase
- Phospho-Acetyl-CoA Carboxylase (Ser79)
- Acetyl-CoA Carboxylase 1
- Acetyl-CoA Carboxylase 2
- ACO2
- ACSL1
- ADH1B
- Adiponectin
- AKR1C2
- Akt (pan)
- Phospho-Akt (Thr308)
- Phospho-Akt (Thr450)
- Phospho-Akt (Ser473)
- Akt1
- Phospho-Akt1 (Ser129)
- Phospho-Akt1 (Ser473)
- Akt2
- Phospho-Akt2 (Ser474)
- Akt3
- Phospho-Akt3 (Ser472)
- ALDH1A1
- Aldolase A
- AMACR
- AMPKα
- Phospho-AMPKα (Thr172)
- AMPKα1
- Phospho-AMPKα1 (Ser485)
- Phospho-AMPKα1 (Ser485) /AMPKα2 (Ser491)
- AMPKα2
- AMPKβ1
- Phospho-AMPKβ1 (Ser108)
- Phospho-AMPKβ1 (Ser182)
- AMPKβ1/2
- AMPKβ2
- AMPKγ1
- AMPKγ2
- AMPKγ3
- α-Amylase
- ANT2/SLC25A5
- AQP2
- Arginase-1
- ARK5
- AS160
- Phospho-AS160 (Ser318)
- Phospho-AS160 (Ser588)
- Phospho-AS160 (Thr642)
- ASCT2
- ATF-4
- ATGL
- ATP Citrate Lyase
- Phospho-ATP Citrate Lyase (Ser455)
- ATPIF1
- BCAT1
- BCAT2
- C1QBP
- CA2
- CA9
- CA12
- CAD
- Phospho-CAD (Ser1859)
- Catalase
- CCTα
- C/EBPβ
- Phospho-C/EBPβ (Thr235)
- C/EBPδ
- CLIC4
- COX IV
- C-Peptide
- CPT1A
- CTMP
- CTR1/SLC31A1
- CYP11A1
- CYP3A4
- DEPTOR/DEPDC6
- DHCR24/Seladin-1
- DJ-1
- DLAT
- DLST
- DPYD
- Enolase-1
- Enolase-2
- eNOS
- Phospho-eNOS (Ser113)
- Phospho-eNOS (Thr495)
- Phospho-eNOS (Ser1177)
- ENPP1
- FAAH1
- FABP1
- FABP4
- Fatty Acid Synthase
- FLCN
- FoxC2
- FoxO1
- Phospho-FoxO1 (Thr24)/FoxO3a (Thr32)
- Phospho-FoxO1 (Thr24)/FoxO3a (Thr32)/FoxO4 (Thr28)
- Phospho-FoxO1 (Ser256)
- Phospho-FoxO1 (Ser319)
- FoxO3a
- Phospho-FoxO3a (Ser253)
- Phospho-FoxO3a (Ser294)
- Phospho-FoxO3a (Ser318/321)
- Phospho-FoxO3a (Ser413)
- FoxO4
- Phospho-FoxO4 (Ser193)
- FTH1
- Fumarase
- Gab1
- Phospho-Gab1 (Tyr307)
- Phospho-Gab1 (Tyr627)
- Phospho-Gab1 (Tyr659)
- Gab2
- Phospho-Gab2 (Ser159)
- Phospho-Gab2 (Tyr452)
- GAPDH
- GβL
- GFAT1
- GFAT2
- GLDC
- Glucagon
- Glucose-6-Phosphate Dehydrogenase
- Glycogen Synthase
- Phospho-Glycogen Synthase (Ser641)
- GPX1
- Phospho-Grb10 (Ser476)
- GSK-3α
- Phospho-GSK-3α (Ser21)
- GSK-3α/β
- Phospho-GSK-3α/β (Ser21/9)
- GSK-3β
- Phospho-GSK-3β (Ser9)
- Phospho-GSK-3β (Thr390)
- hERG1a
- Hexokinase I
- Hexokinase II
- HO-1
- HMOX2/HO-2
- HSL
- Phospho-HSL (Ser563)
- Phospho-HSL (Ser565)
- Phospho-HSL (Ser660)
- IDH1
- IDH2
- IGF-I Receptor β
- Phospho-IGF-I Receptor β (Tyr980)

Phospho-IGF-I Receptor β (Tyr1131)
Phospho-IGF-I Receptor β (Tyr1131)/Insulin Receptor β (Tyr1146)
Phospho-IGF-I Receptor β (Tyr1135)
Phospho-IGF-I Receptor β (Tyr1135/1136)/Insulin Receptor β (Tyr1150/1151)
Phospho-IGF-I Receptor β (Tyr1316)
IGFBP2
Insulin Receptor β
Phospho-Insulin Receptor β (Tyr1146)
Phospho-Insulin Receptor β (Tyr1150/1151)
Phospho-Insulin Receptor β (Tyr1345)
Phospho-Insulin Receptor β (Tyr1361)
Insulin
IRAP
IRS-1
Phospho-IRS-1 (panTyr)
Phospho-IRS-1 (Ser302)
Phospho-IRS-1 (Ser307)
Phospho-IRS-1 (Ser318)
Phospho-IRS-1 (Ser332/336)
Phospho-IRS-1 (Ser612)
Phospho-IRS-1 (Ser636/639)
Phospho-IRS-1 (Ser789)
Phospho-IRS-1 (Tyr895)
Phospho-IRS-1 (Ser1101)
Phospho-IRS-1 (Tyr1222)
IRS-2
Phospho-IRS-2 (panTyr)
LAMTOR1/C11orf59
LAMTOR2/ROBLD3
LAMTOR3/MAPKSP1
LAMTOR4/C7orf59
LARS
LAT1
LDHA
Phospho-LDHA (Tyr10)
LDHA/LDHC
LIPIN1
LKB1
Phospho-LKB1 (Thr189)
Phospho-LKB1 (Ser334)
Phospho-LKB1 (Ser428)
LXR-β
Malic Enzyme
MDH2
Mios
Mitofusin-1
Mitofusin-2
MO25α/CAB39
MPC1
MRP2/ABCC2
MTAP
mTOR
Phospho-mTOR (Ser2448)
Phospho-mTOR (Ser2481)
NBC1/SLC4A4
NME1/NDKA
NPC1L1
NQO1
NRF1
OGDH
OGT
p70 S6 Kinase
Phospho-p70 S6 Kinase (Thr371)
Phospho-p70 S6 Kinase (Thr389)
Phospho-p70 S6 Kinase (Thr389/Thr421/Ser424)
Phospho-p70 S6 Kinase (Thr421/Ser424)
p70 S6 Kinase 2
PANK4
PASK
PCK1
PCK2
PDHK1
PDK1
Phospho-PDK1 (Ser241)
Pdx1
Perilipin
PFKFB2
Phospho-PFKFB2 (Ser483)
PFKFB3
PFKL
PFKP
PGAM1
PGC-1α
PGD
PHGDH
PI3 Kinase p55
PI3 Kinase p85α
Phospho-PI3 Kinase p85α (Tyr458)/p55 (Tyr199)
PI3 Kinase p101
PI3 Kinase p110α
PI3 Kinase p110β
PI3 Kinase p110γ
PI3 Kinase Class II α
PI3 Kinase Class III
PI4 Kinase
PiT1/SLC20A1
PKM1
PKM1/2
PKM2
Phospho-PKM2 (Tyr105)
PRAS40
Phospho-PRAS40 (Ser183)
Phospho-PRAS40 (Thr246)
Prdx1
Phospho-Prdx1 (Tyr194)
Protor2
PTEN
Phospho-PTEN (Ser380)
Phospho-PTEN (Ser380/Thr382/383)
Non-phospho-PTEN (Ser380/Thr382/383)
PTP1B
Pyruvate Dehydrogenase
RagA
RagB
RagC
RagD
Raptor
Phospho-Raptor (Ser792)
REDD1
Rheb
Rictor
Phospho-Rictor (Thr1135)
SCAP
SCD1
SDHA
SGK1
Phospho-SGK1 (Ser78)
SGK2
SGK3
Phospho-SGK3 (Thr320)
SGLT1
SGLT2
SHMT1
SHMT2
SIK2
Sin1
Phospho-Sin1 (Thr86)
SNARK/NUAK2
SOD2
StAR
Succinyl-CoA Synthetase
Synip
Thioredoxin 1
Thioredoxin 2
Thymidine Kinase
Thymidylate Synthase
Tom20
TORC2/CRTC2
Transketolase
TRXR1
TRXR2/TXNRD2
Hamartin/TSC1
Tuberin/TSC2
Phospho-Tuberin/TSC2 (Ser939)
Phospho-Tuberin/TSC2 (Ser1254)
Phospho-Tuberin/TSC2 (Ser1387)
Phospho-Tuberin/TSC2 (Thr1462)
Phospho-Tuberin/TSC2 (Tyr1571)
Tug
Tyrosinase
xCT/SLC7A11

AMPK Signaling and Lipid Metabolism



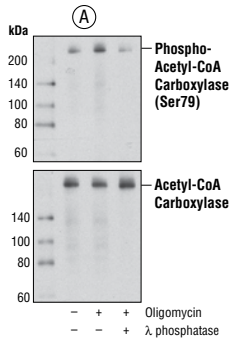
AMP-activated protein kinase (AMPK) is a serine/threonine kinase that acts as a central regulator of energy metabolism. AMPK is activated by increases in the ratio of AMP and ADP to ATP as a result of cellular or environmental stresses such as low glucose, hypoxia, heat shock, or ischemia. Active AMPK positively regulates signaling pathways that replenish ATP supplies—such as insulin-stimulated glucose uptake via Glut4, increased fatty acid oxidation by ACC, or increased glycolysis by PFK2. Concurrently, AMPK negatively regulates proteins central to ATP-consuming processes such as mTORC1, glycogen synthase, SREBP-1, and TSC2, resulting in down-regulation of gluconeogenesis and inhibition of glycogen, lipid, and protein synthesis.

AMPK plays a role in many human diseases. As a critical mediator of glucose and lipid metabolism, AMPK is an important therapeutic target for type 2 diabetes (T2D), a disease characterized by insulin resistance and inadequate glucose uptake. Several direct and indirect AMPK activators, such as metformin, resveratrol, and the thiazolidinediones (TZDs), are currently prescribed for T2D to reduce blood glucose levels and increase insulin sensitivity. In the heart, AMPK regulates cardiac metabolism and the ATP production necessary to maintain normal cardiac contraction. AMPK has been found to have a protective effect during cardiac stress by regulating ATP levels after minor ischemic injury. In addition, mutations in the AMPKγ2 subunit are associated with Wolff-Parkinson-White syndrome, a form of heart disease commonly associated with hypertrophy and excessive glycogen storage in cardiac myocytes. AMPK also plays a role in cancer due to the fact that its upstream kinase, LKB1, is a tumor suppressor. Loss-of-function mutations in LKB1 have been identified in many cancers, including non-small cell lung cancer, cervical cancer, and melanomas. AMPK's ability to restrict protein synthesis and cell growth through inhibition of mTORC1 makes it an attractive therapeutic target for cancer. In support of this, some AMPK activators such as metformin have been shown to delay tumor onset or be associated with a lower rate of tumorigenesis.

Select Reviews: Coughlan, K.A., Valentine, R.J., Ruderman, N.B., and Saha, A.K. (2014) *Diabetes Metab. Syndr. Obes.* 7, 241–253. Hardie, D.G. (2014) *Curr. Opin. Cell Biol.* 33C, 1–7. Hardie, D.G. (2014) *J. Intern. Med.* 276, 543–559. Kim, T.T. and Dyck, J.R. (2015) *Trends Endocrinol. Metab.* 26, 40–48.

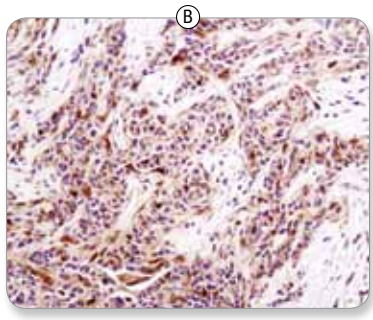
TARGET LOCALIZATION

Primary antibodies to assess key protein targets within the AMPK pathway



Phospho-Acetyl-CoA Carboxylase (Ser79) (D7D11) Rabbit mAb #11818: WB analysis of extracts from SH-SY5Y cells **(A)**, untreated or treated with Oligomycin #9996 (0.5 µM, 30 min), using #11818 (upper) or Acetyl-CoA Carboxylase (C83B10) Rabbit mAb #3676 (lower). The phospho-specificity of the antibody was verified by λ phosphatase treatment.

LKB1 (D60C5F10) Rabbit mAb (IHC Formulated) #13031: IHC analysis of paraffin-embedded human ovarian carcinoma **(B)** using #13031.

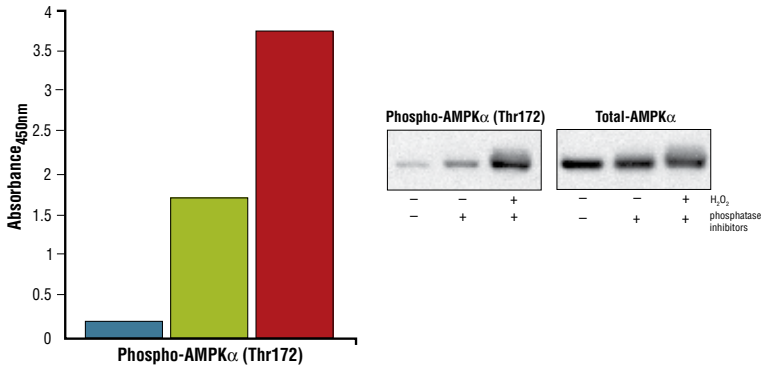


QUANTITATIVE ASSAYS

PathScan® Sandwich ELISA Kits for quantitative analysis

PathScan® Phospho-AMPKα (Thr172) Sandwich ELISA Kit #7959: Treatment of C2C12 cells with H_2O_2 stimulates phosphorylation of AMPKα at Thr172, detected by #7959. C2C12 cells (80–90% confluent) were untreated or treated with H_2O_2 (10 mM, 10 min, 37°C). The absorbance readings at 450 nm are shown in the left figure, while the corresponding western blots using AMPKα (23A3) Rabbit mAb #2603 (right panel) or Phospho-AMPKα (Thr172) (D79.5E) Rabbit mAb #4188 (left panel) are shown in the right figure.

Control without phosphatase inhibitor
Control
H_2O_2-treated



ANTIBODY SAMPLER KITS

For convenient investigation of multiple targets within a single pathway. Visit our website for a complete listing of Antibody Sampler Kits.

#12589 Adipogenesis Marker Antibody Sampler Kit

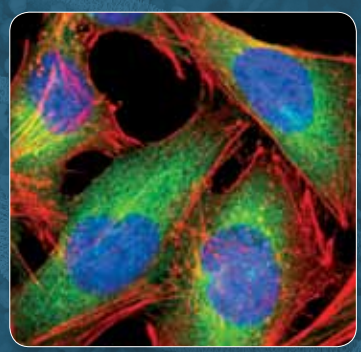
#9957 AMPK and ACC Antibody Sampler Kit

#8335 Fatty Acid and Lipid Metabolism Antibody Sampler Kit

#8350 Phospho-TSC2 Antibody Sampler Kit

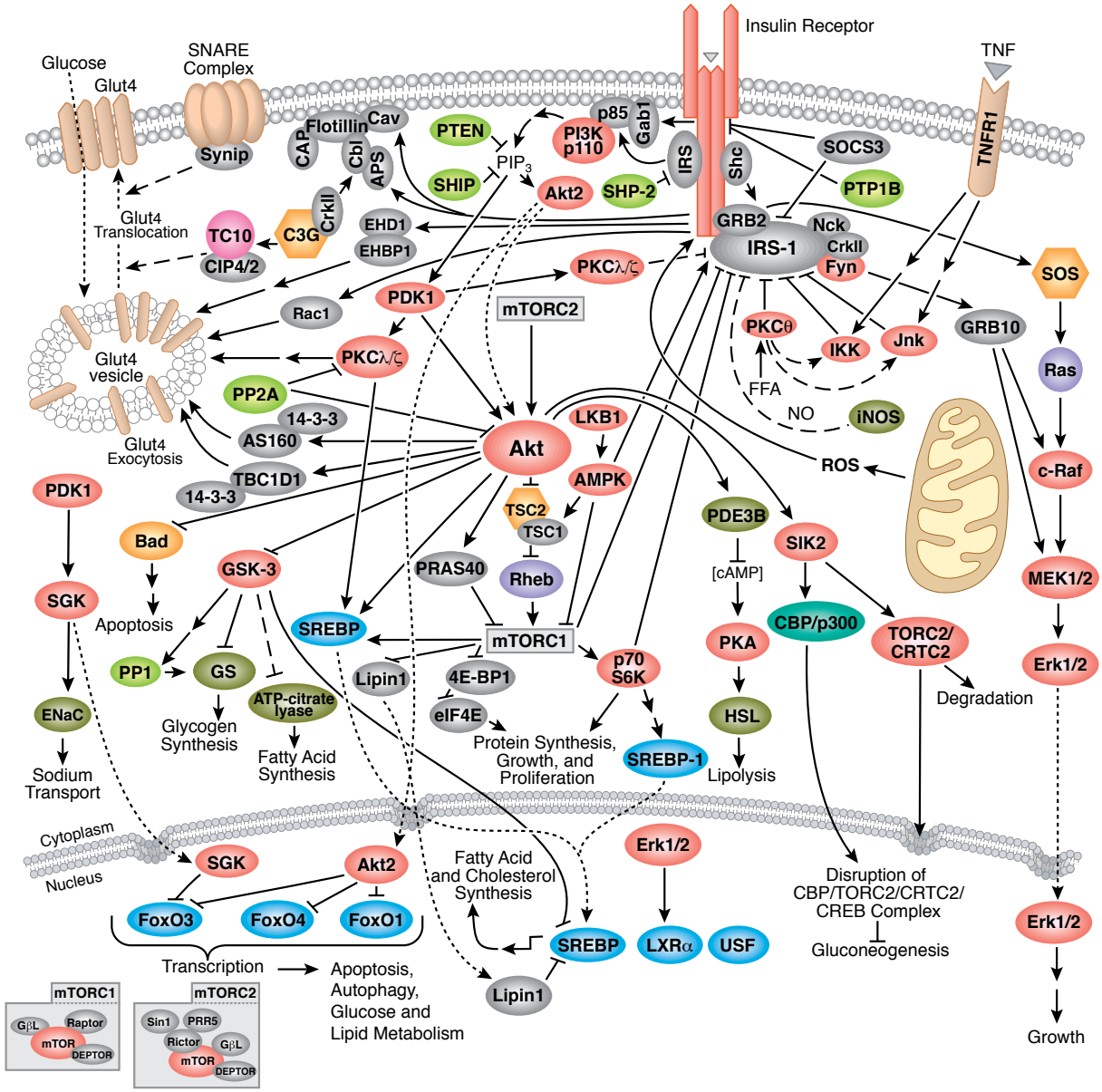
Lipid Metabolism

Acetyl-CoA is produced in the mitochondria during the metabolic breakdown of both glucose and fatty acids. Newly generated acetyl-CoA combines with oxaloacetate to produce citrate, which progresses through the Krebs cycle to generate ATP and replenish Krebs cycle intermediates. When fatty acid production is necessary, citrate can exit the mitochondria and be converted back into acetyl-CoA in the cytosol through the action of ATP-citrate lyase (ACL). Acetyl-CoA carboxylase (ACC) then catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, the rate-limiting step in fatty acid synthesis. This reaction is tightly controlled through inhibitory phosphorylations by AMPK and PKA, linking synthesis of new fatty acids to nutrient availability. Fatty acid synthesis is mediated through the action of fatty acid synthase (FAS). The fatty acids produced through these reactions are the building blocks for triglycerides, which are eventually exported to adipocytes and stored to meet future energy demands.



Fatty Acid Synthase (C20G5) Rabbit mAb #3180: Confocal IF analysis of HeLa cells using #3180 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® (fluorescent DNA dye).

Insulin Signaling and Glucose Transport



Pancreatic β-cells work to maintain glucose homeostasis by releasing insulin into the blood stream in response to glucose-sensing pathways throughout the body. Insulin stimulates glucose uptake from blood into skeletal muscle, cardiac muscle, and adipose tissue through a signaling cascade mediated by the insulin receptor (IR). Insulin binding to the IR results in activation of the insulin receptor substrate (IRS) protein and subsequent signaling to the PI3K/Akt and Erk1/2 pathways, resulting in translocation of Glut4 from intracellular vesicles to the plasma membrane, glucose uptake, cell proliferation, and survival. Liver is also an insulin-sensitive tissue that takes up glucose via Glut2.

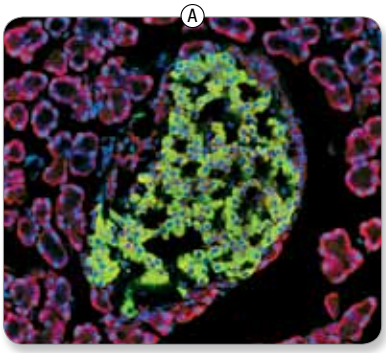
Aberrant insulin signaling is most often linked with type 2 diabetes (T2D), a disease characterized by insulin resistance and inadequate glucose uptake affecting nearly 350 million people worldwide. Current treatment strategies fall into a number of classes based on their mechanism of action: insulin analogs (e.g., insulin glargine); direct and indirect AMPK activators (e.g., metformin, resveratrol, and the thiazolidinediones); inducers of β-cell insulin release (e.g., sulfonylureas and GLP-1 receptor agonists); and exercise, which directly increases Glut4 translocation and glucose uptake in skeletal muscle. Insulin resistance tends to be inextricably linked with obesity; elevated insulin levels induce fat storage in adipocytes and lead to weight gain, while increased fat deposits secrete TNFα and other inflammatory cytokines that further induce insulin resistance. Insulin resistance is also linked to elevated serum triglycerides, hypertension, and high HDL cholesterol levels, which together form the defining characteristics of metabolic syndrome. In addition, insulin resistance is also associated with cardiovascular disease, due to the effect of insulin on vasodilation.

The insulin-like growth factor-I receptor (IGF-IR) is similar in structure to the IR and can exist as either a homodimer or as an IGF-IR/IR heterodimer. IGF-IR uses a similar signaling cascade to promote cell proliferation and survival. IGF-IR overexpression has been identified in many forms of cancer and constitutive signaling has been found to be an important contributor to the initiation and growth of tumor cells.

Select Reviews: Al-Goblan, A.S., Al-Alfi, M.A., and Khan, M.Z. (2014) *Diabetes Metab. Syndr. Obes.* 7, 587–591. Du, Y. and Wei, T. (2014) *Protein Cell* 5, 203–213. International Diabetes Federation (2013) *Diabetes Atlas, sixth edition www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf* Janssen, J.A. and Varewijck, A.J. (2014) *Front. Endocrinol.* 5, 224. Richter, E.A. and Hargreaves, M. (2013) *Physiol. Rev.* 93, 993–1017. Shin, J.A., Lee, J.H., and Lim, S.Y. et al. (2013) *J. Diabetes Investig.* 4, 334–343.

TARGET LOCALIZATION

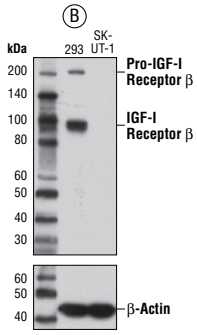
Primary antibodies to assess localization and expression of key targets

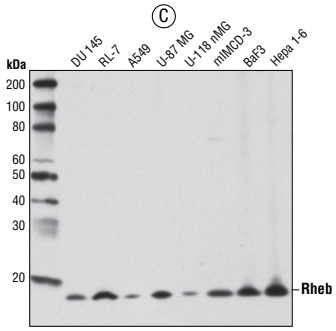


Insulin (L6B10) Mouse mAb #8138: Confocal IF analysis of rat pancreas **(A)** using #8138 (green) and S6 Ribosomal Protein (5G10) Rabbit mAb #2217 (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).

IGF-I Receptor β (D23H3) XP® Rabbit mAb #9750: WB analysis of extracts from 293 (IGF-I receptor β+) and SK-UT-1 (IGF-I receptor β-) cells **(B)**, using #9750 (upper) or β-Actin Antibody #4967 (lower).

Rheb (E1G1R) Rabbit mAb #13879: WB analysis of extracts from various cell lines **(C)** using #13879.



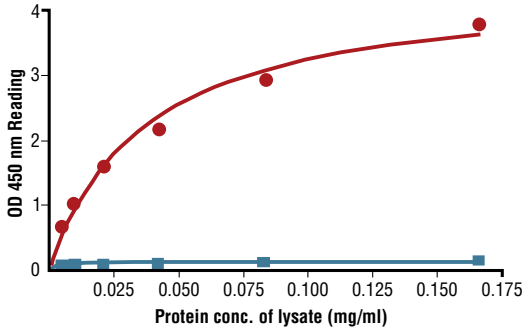


QUANTITATIVE ASSAYS

PathScan® Sandwich ELISA Kits for quantitative analysis

PathScan® Phospho-Insulin Receptor β (Tyr1150/1151) Sandwich ELISA Kit #7258: The relationship between protein concentration of lysates from untreated and insulin-treated CHO-IR/IRS-1 cells and kit assay optical density readings is shown. After starvation, CHO-IR/IRS-1 cells (85% confluence) were treated with insulin (100 nM, 2 min, 37°C) and then lysed. CHO-IR/IRS-1 cells stably overexpress human insulin receptor and rat IRS-1.

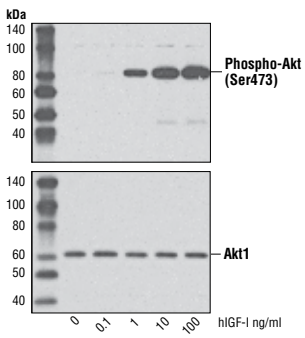
● **Insulin-treated**
■ **Untreated**



COMPANION REAGENTS

Recombinant growth factors to induce signaling

Human Insulin-like Growth Factor I (hIGF-I) #8917: WB analysis of extracts from MCF-7 cells, untreated or treated with hIGF-I for 10 min, using Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060 (upper) and Akt1 (C73H10) Rabbit mAb #2938 (lower).



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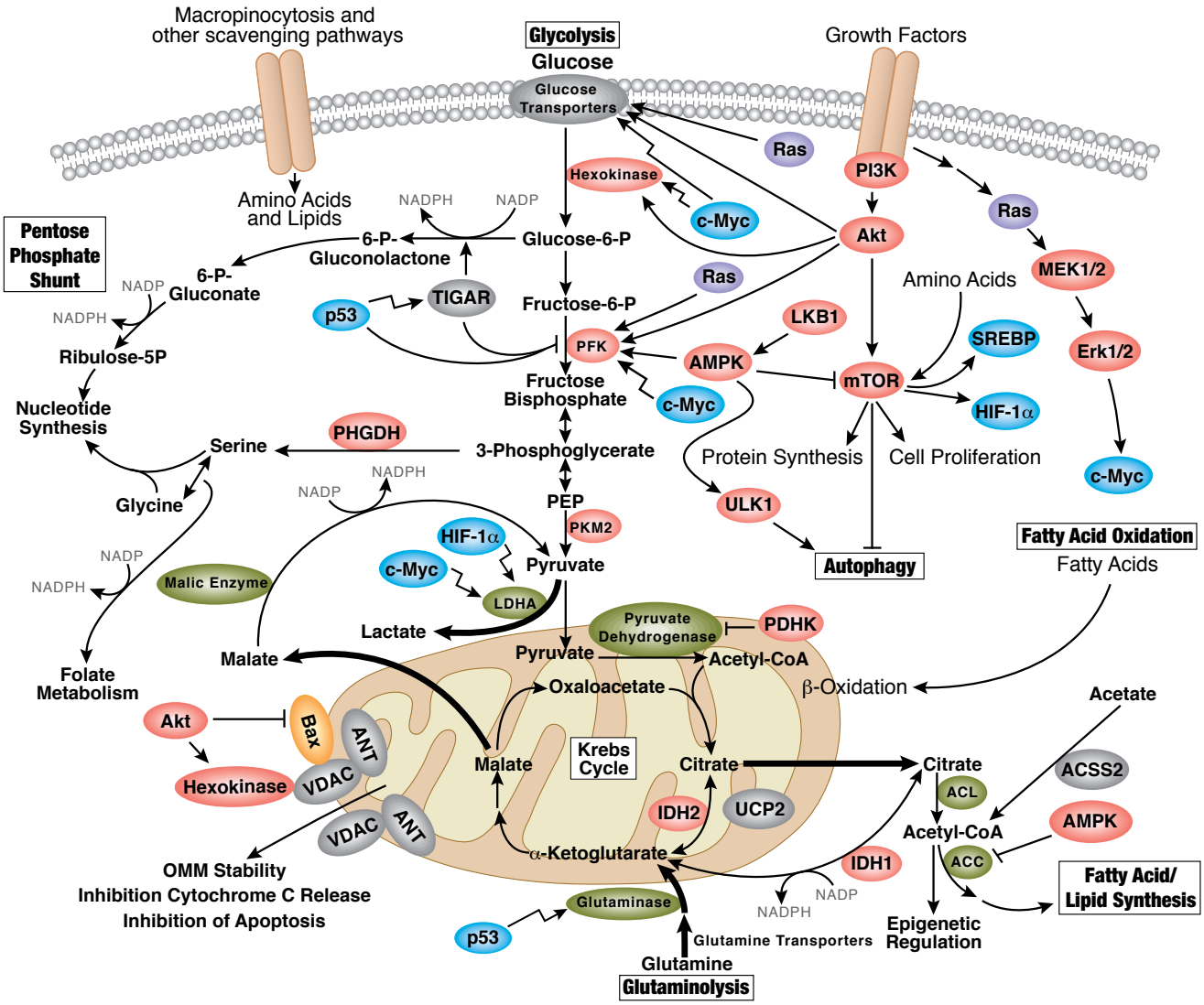
#3015 Insulin Receptor Substrate Antibody Sampler Kit
#12879 IRS-1 Inhibition Antibody Sampler Kit

Warburg Effect



Normal cells typically generate ATP by metabolizing glucose via glycolysis followed by oxidative phosphorylation in the mitochondria. However, in the 1920s, Otto Warburg observed that tumor cells produce energy by unusually high rates of glycolysis and lactate fermentation (without subsequent cycling through oxidative phosphorylation). This phenomenon is known as aerobic glycolysis or the Warburg effect. In addition to high glycolytic rates, tumor cells also display an increased dependence on glutamine metabolism, fatty acid synthesis, and the pentose phosphate shunt (PPS), which together produce the nucleotides, lipids, and Krebs cycle intermediates necessary for rapid cell growth.

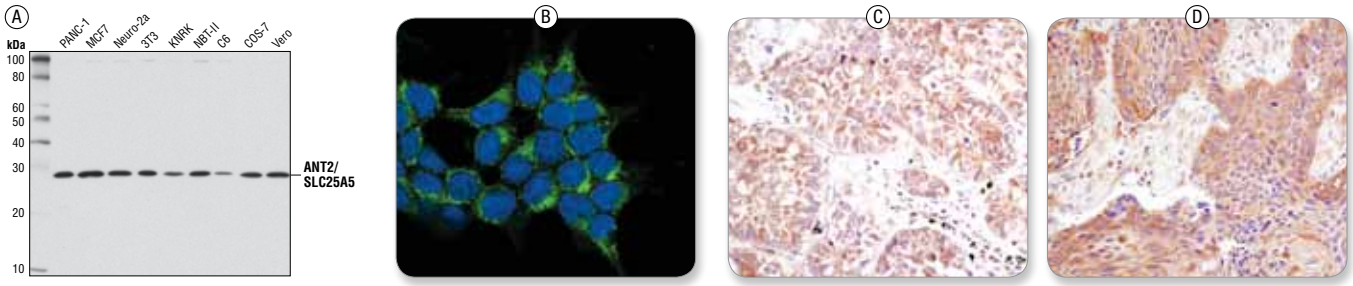
The altered metabolism of cancer cells is controlled by aberrant expression of oncogenes and tumor suppressors within several signaling pathways that regulate the transcription and activity of key metabolic enzymes. For example, the Akt, Erk1/2, and AMPK pathways control the activity (while Myc regulates the transcription) of several glycolytic enzymes such as hexokinase, phosphofructokinase (PFK), and pyruvate kinase isoform M2 (PKM2). In addition, p53 is a transcriptional regulator of targets important for glutaminolysis and the PPS.

Because ATP generation by glycolysis alone produces significantly less ATP per glucose molecule than oxidative phosphorylation, tumor cells consume and metabolize large quantities of glucose. This glucose "addiction" has been targeted by researchers as a point of therapeutic intervention. A number of metabolism-targeting drugs are currently under clinical consideration, including glucose transporter inhibitors, glucose mimetics such as 2-DG that are unable to be metabolized, the AMPK inhibitor metformin, and inhibitors of the glycolytic enzymes hexokinase II, PFK, PKM2, and LDH-A. Use of these agents is currently being evaluated alone and in combination with chemotherapy or other targeted, small-molecule inhibitors.

Select Reviews: Elf, S.E. and Chen, J. (2014) *Cancer* 120, 774–780. Jang, M., Kim, S.S., and Lee, J. (2013) *Exp. Mol. Med.* 45, e45. Kee, H.J. and Cheong, J.H. (2014) *BMB Rep.* 47, 158–166. Zhang, Y. and Yang, J.M. (2013) *Cancer Biol. Ther.* 14, 81–89.

TARGET LOCALIZATION

Primary antibodies to investigate the expression or localization of key metabolic enzymes

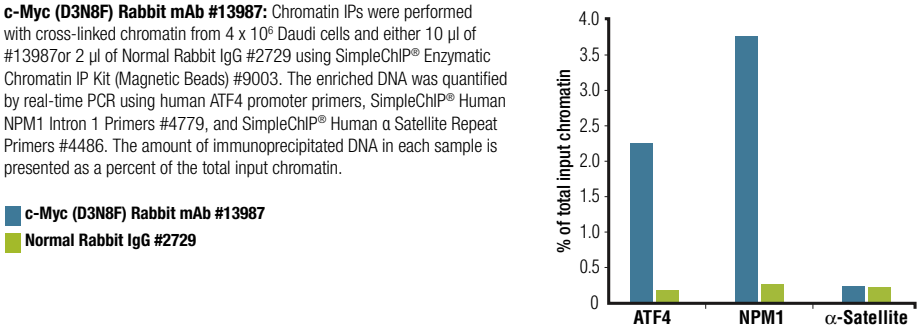


ANT2/SLC25A5 (E2B9D) Rabbit mAb #14671: WB analysis of extracts from various cell lines **(A)** using #14671. **IDH1 (D2H1) Rabbit mAb #8137:** Confocal IF analysis of 293T cells **(B)** using #8137 (green). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye). **Hexokinase II (C64G5) Rabbit mAb #2867:** IHC analysis of paraffin-embedded human lung carcinoma **(C)** using #2867. **PKM2 (D78A4) XP® Rabbit mAb #4053:** IHC analysis of paraffin-embedded human lung carcinoma **(D)** using #4053.

QUANTITATIVE ASSAYS

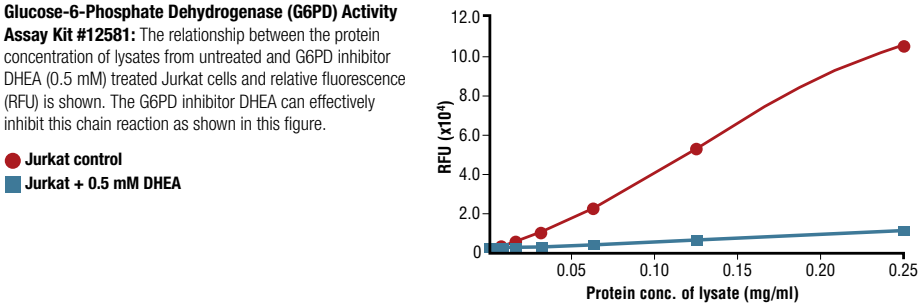
ChIP-validated kits, primers, and antibodies to examine protein-DNA interactions

c-Myc (D3N8F) Rabbit mAb #13987: Chromatin IPs were performed with cross-linked chromatin from 4 x 10^6 Daudi cells and either 10 µl of #13987or 2 µl of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using human ATF4 promoter primers, SimpleChIP® Human NPM1 Intron 1 Primers #4779, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is presented as a percent of the total input chromatin.

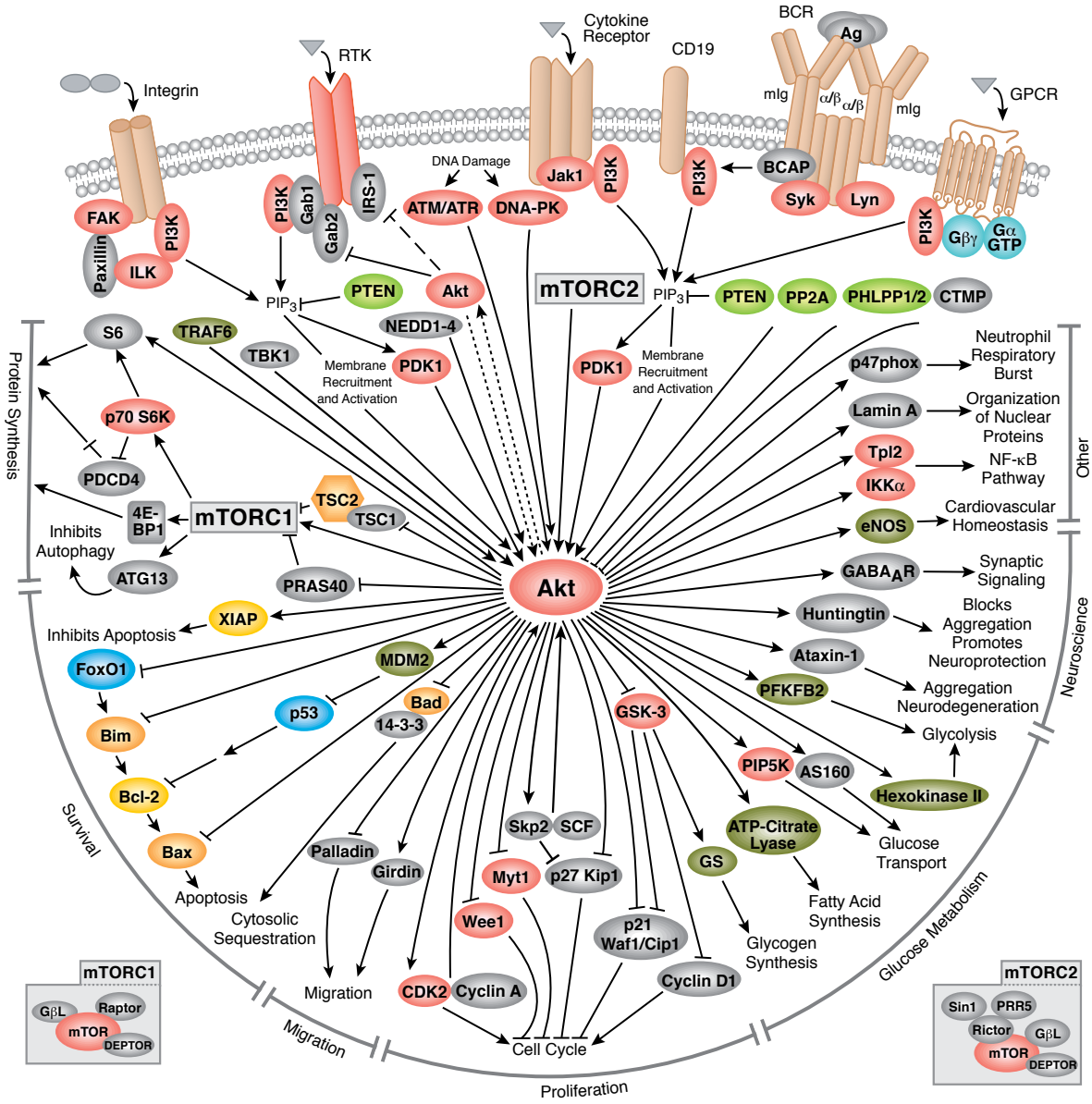


Glucose-6-Phosphate Dehydrogenase Activity Assay Kit to assess the rate-limiting step in the pentose phosphate pathway

Glucose-6-Phosphate Dehydrogenase (G6PD) Activity Assay Kit #12581: The relationship between the protein concentration of lysates from untreated and G6PD inhibitor DHEA (0.5 mM) treated Jurkat cells and relative fluorescence (RFU) is shown. The G6PD inhibitor DHEA can effectively inhibit this chain reaction as shown in this figure.



Akt Signaling



The serine/threonine kinase Akt exists as three isoforms in mammals. Akt1 and Akt2 have a widespread tissue distribution, whereas Akt3 is expressed in testes and brain. Akt regulates many important biological processes including cell survival, proliferation, growth, and glycogen metabolism. Various growth factors, hormones, and cytokines activate Akt by binding their cognate receptor tyrosine kinase (RTK), cytokine receptor, or G protein-coupled receptor (GPCR). This triggers activation of the lipid kinase PI3K, which generates PIP3 to draw Akt to the plasma membrane. Akt is then activated through dual phosphorylation at Thr308 by PDK1 and Ser473 by mTORC2.

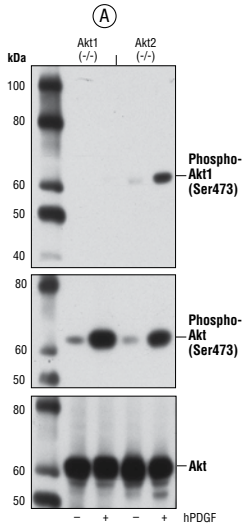
Akt phosphorylates a large number of downstream targets that mediate its metabolic and mitogenic effects. Akt plays an important role in signaling through the insulin receptor, and inappropriate Akt activation can result in the insulin resistance associated with type 2 diabetes (T2D), metabolic syndrome, and some forms of obesity. Akt directly mediates the effects of insulin, including Glut4 translocation and glucose uptake (through phosphorylation of AS160 and TBC1D1), activation of protein synthesis and growth via mTORC1, and inhibition of gluconeogenesis in the liver. Akt also regulates general glucose and lipid metabolism. Active Akt phosphorylates GSK-3 to increase glycogen synthesis, the enzymes hexokinase and phosphofructokinase to increase glycolysis, and ATP-citrate lyase to increase fatty acid synthesis.

The profound effects of Akt on cell proliferation and survival have made it a central focus of cancer research. Akt inhibits the cell cycle inhibitors Wee1, p27 Kip1, and p21 Cip1 to promote cell proliferation. Akt prevents apoptosis through inhibition of several pro-apoptotic factors such as Bim, Bax, Bad, and FoxO1. Akt also activates the apoptosis inhibitor protein, XIAP, and blocks p53-mediated apoptosis through stabilization of MDM2. Activating mutations in PI3K3C (a subunit of PI3K), and loss-of-function mutations in PTEN both upregulate Akt activity and are some of the most commonly mutated genes in cancer.

Select Reviews: Beretta, M., Bauer, M., and Hirsch, E. (2014) *Adv. Biol. Regul.* Dec. 4 *[ePub ahead of print]*. Guo, S. (2014) *J. Endocrinol.* 220, T1–T23. Hers, I., Vincent, E.E., and Tavaré, J.M. (2011) *Cell Signaling* 23, 1515–1527. Toker, A. and Marmiroli, S. (2014) *Adv. Biol. Regul.* 55, 28–38.

TARGET LOCALIZATION

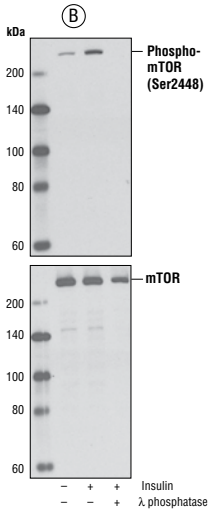
Primary antibodies to assess localization and expression of Akt and its targets

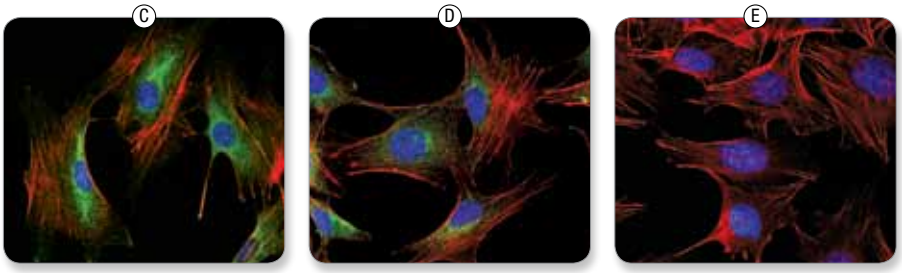


Phospho-Akt1 (Ser473) (D7F10) XP® Rabbit mAb (Akt1 Specific) #9018: WB analysis of extracts from Akt1 (-/-) mouse embryonic fibroblasts (MEFs) or Akt2 (-/-) MEFs, untreated or stimulated with hPDGF #8913 (100 ng/ml, 15 min), **(A)** using #9018 (upper), Phospho-Akt (Ser473) (D9E) XP® Rabbit mAb #4060 (middle), or Akt (pan) (C67E7) Rabbit mAb #4691 (lower).

Phospho-mTOR (Ser2448) (D9C2) XP® Rabbit mAb #5536: WB analysis of extracts from serum-starved NIH/3T3 cells, untreated or insulin-treated (150 nM, 5 min), alone or in combination with λ-phosphatase **(B)**, using #5536 (upper) or mTOR (7C10) Rabbit mAb #2983 (lower).

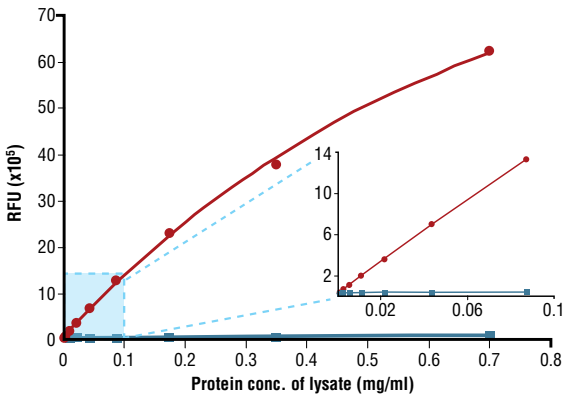
GSK-3β (D5C5Z) XP® Rabbit mAb #12456: Confocal IF analysis of wild-type mouse embryonic fibroblasts (MEFs) **(C)**, GSK-3α (-/-) MEFs **(D)**, and GSK-3β (-/-) MEFs **(E)** using #12456 (green). Actin filaments were labeled with DY-554 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye). (MEF wild type, GSK-3α (-/-), and GSK-3β (-/-) cells were kindly provided by Dr. Jim Woodgett, University of Toronto, Canada).





QUANTITATIVE ASSAYS

PathScan® Sandwich ELISA Kits for quantitative analysis of Akt activation



PathScan® Phospho-Akt (Thr308) Chemiluminescent Sandwich ELISA Kit #7135: Relationship between protein concentration of lysates from untreated and PDGF-treated NIH/3T3 cells and immediate light generation with chemiluminescent substrate is shown. Cells (at 80% confluence) were treated with PDGF (50 ng/ml) and lysed after incubation at 37°C for 5 min. Graph inset corresponding to the shaded area shows high sensitivity and a linear response at the low protein concentration range.

● **PDGF-treated**
■ **Untreated**

Troy, Product Scientist, has been with CST since 2010.

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