Research Focus

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# New! PI 3-Kinase/Akt Signaling Research Tools

PI 3-kinases are ubiquitous, heterodimeric lipid kinases that regulate cell growth, motility, proliferation, survival and other processes. These dual-specificity enzymes phosphorylate phosphoinositides. The family of eight PI 3-kinases is divided into four classes, which differ in their substrate selectivity and regulation.

## Molecular mechanisms of PI 3-K signaling

Activated PI 3-kinase phosphorylates phosphoinositol (PI) substrates to produce PI(3)P, PI(3,4)$P_2$, and PI(3,4,5)$P_3$. These molecules act as second messengers and recruit the PI 3-K-dependent serine/threonine kinase (PDK1) and Akt from the cytoplasm to the plasma membrane. Lipid binding and membrane translocation lead to conformational changes in Akt, which gets phosphorylated on $Thr^{308}$ in the activation loop by PDK1 and on $Ser^{473}$ in the hydrophobic phosphorylation motif by mTORC2. This dual phosphorylation causes full activation of the enzyme. Inhibitors of PI 3-kinase and overexpression of dominant negative PI 3-kinase mutants block many cellular responses to insulin, indicating that PI 3-kinase lies upstream of these events.

## Dysregulated PI 3-kinase signaling and disease

Dysregulated PI 3-kinase signaling has been reported in a variety of human tumors. Over 30% of solid tumors are reported to contain mutations in the catalytic unit of PI 3-kinase. Functional analyses of the catalytic subunit of mutated PI 3-kinases indicate that these mutations increase its enzymatic activity above normal, stimulate Akt signaling, allow growth factor-independent growth and promote cell invasion and metastasis. Hence, PI 3-kinase is becoming an attractive target for drug development, not only in the areas of cancer and other proliferative diseases, but also in the treatment of diabetes, inflammation, and immune disorders.

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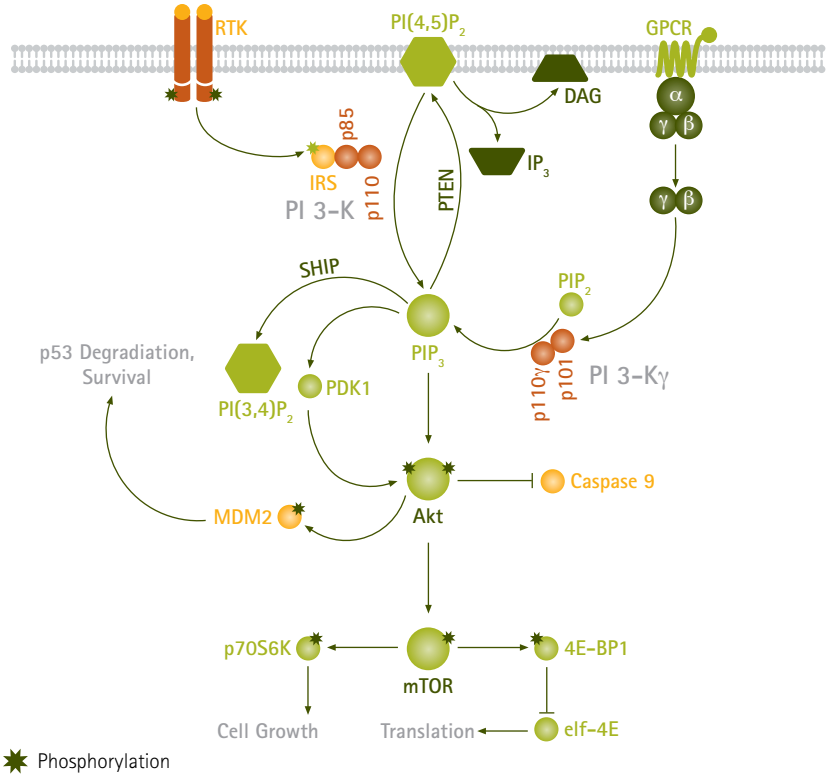



**Figure 1.**

Activation of the PI 3-kinase pathway by growth and survival signals at the cell surface causes signal transduction via second messengers, Akt and mTOR through the cytoplasm to the nucleus, and eventually promotes protein translation, cell growth and survival.

## Akt, a central protein in the PI 3-kinase pathway

Akt (protein kinase B), a serine/threonine kinase, is a critical enzyme in several signal transduction pathways involved in cell proliferation, apoptosis, angiogenesis and diabetes. In mammals, three highly homologous isoforms of Akt have been reported (α, β, γ or Akt 1, 2, 3), which differ slightly in their regulatory phosphorylation sites. Akt1 plays an important role in growth and survival and Akt2 acts primarily as a regulator of glucose metabolism. Although Akt3 does not contribute significantly to the maintenance of normal metabolism in tissues, it is essential for the attainment of normal organ size, probably influencing both cell size and number via mTOR activation. Activation of Akt involves growth factor binding to a receptor tyrosine kinase and subsequent activation of PI 3-kinase, which phosphorylates membrane-bound PIP2 ($PI(4,5)P_2$ in Figure 1) to generate PIP3. The binding of PIP3 to the pleckstrin homology (PH) domain of Akt anchors Akt to the plasma membrane. Akt is activated following its phosphorylation at $Thr^{308}$ on the kinase domain by PDK1 and on $Ser^{473}$ on the hydrophobic motif by mTORC2.

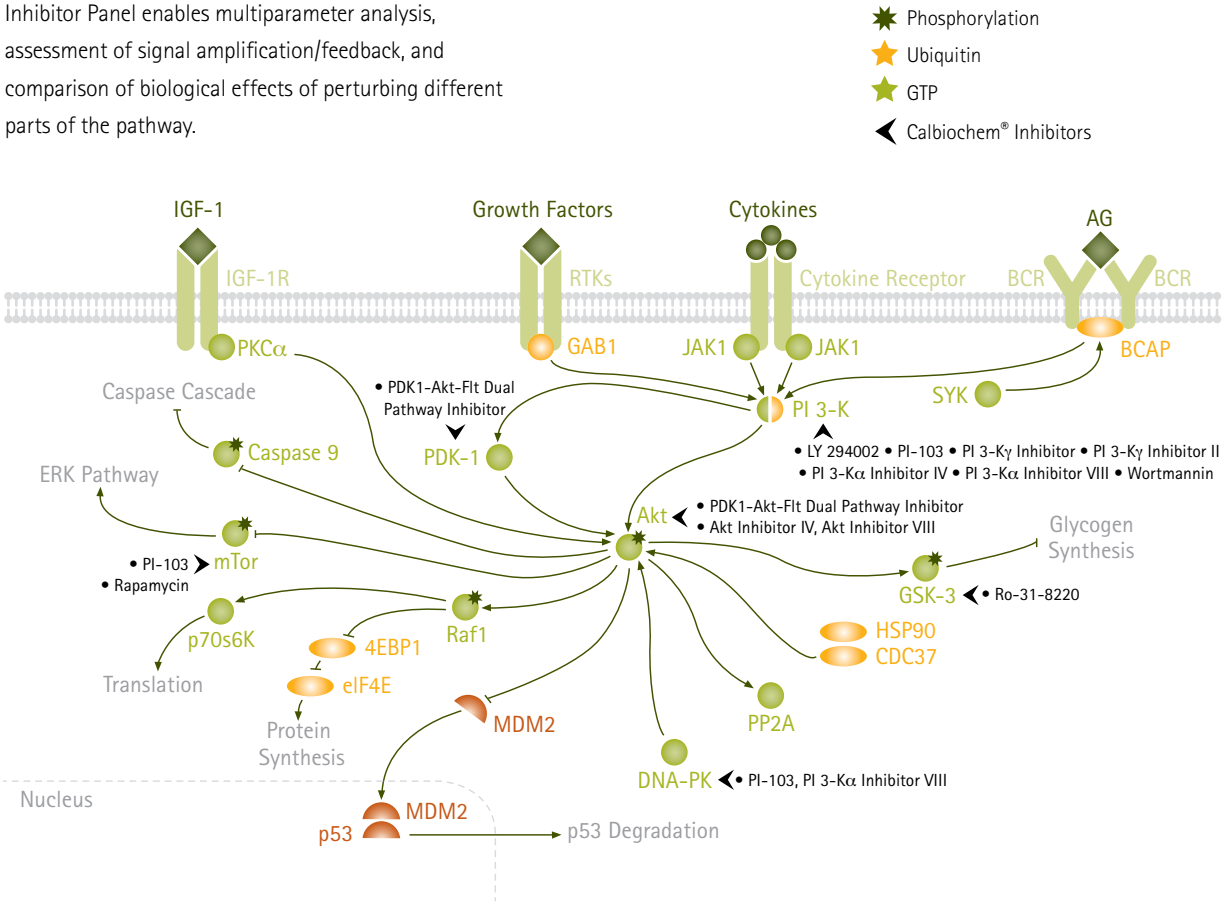
## Mechanism of Akt-mediated survival via GSK-3

The principal roles of Akt are to facilitate growth factor-mediated cell survival and to block apoptotic cell death. Akt achieves this by phosphorylating a variety of substrates, such as Bad, MDM2, caspase-9, Forkhead transcription factors and GSK-3. Insulin receptors in all insulin-sensitive tissues are coupled to Akt, which phosphorylates and inactivates GSK-3. The activity of GSK-3 is inhibited by N-terminal serine phosphorylation of the two GSK-3 isoforms, $Ser^{9}$ in GSK-3β and $Ser^{21}$ in GSK-3α. Hence, insulin increases the phosphorylation of both Akt and GSK-3. Under conditions of diabetes and insulin resistance, Akt and GSK-3 are both usually dephosphorylated in adipose tissue and skeletal muscle, resulting in a lack of glycogen synthase activity and reduced glucose metabolism.

# InhibitorSelect™ PI 3-K/Akt/mTOR Signaling Pathway Inhibitor Panel

**(Catalogue No. 124031)**

The InhibitorSelect™ PI 3-K/Akt/mTOR Signaling Pathway Inhibitor Panel enables multiparameter analysis, assessment of signal amplification/feedback, and comparison of biological effects of perturbing different parts of the pathway.



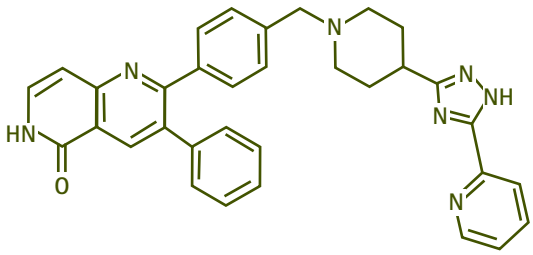

| Description | Target | Catalogue No. |
|---|---|---|
| Akt Inhibitor IV | Akt | 124011 |
| Akt Inhibitor VIII, Isozyme-Selective, Akti-1/2 | Akt1, Akt2 | 124018 |
| LY 294002 | PI 3-K | 440202 |
| LY 303511 | Negative control for LY 294002 | 440203 |
| PDK1/Akt/Flt Dual Pathway Inhibitor | Akt, Flt, PDK1 | 521275 |
| PI-103 | DNA-PK, PI 3-K, mTOR | 528100 |
| PI 3-Kγ Inhibitor | PI 3-Kγ | 528106 |
| PI 3-Kγ Inhibitor II | PI 3-Kγ | 528108 |
| PI 3-Kα Inhibitor IV | PI 3-Kα + β | 528111 |
| PI 3-Kα Inhibitor VIII | DNA-PK, PI 3-K | 528116 |
| Rapamycin | mTOR, p70S6 | 553210 |
| Ro-31-8220 | PKC, GSK-3 | 557520 |
| Wortmannin | PI 3-K Irreversible | 681675 |

## Akt Inhibitor XV, Isozyme-Selective

**(5-Hydroxy-3-phenyl-2-(4-((4-(5-pyridin-2-yl-1H-1,2,4-triazol-3-yl)piperdin-1-yl)methyl) phenyl)-1,6-naphthyridine)**

**(Qty: 2 mg, Catalogue No. 124034)**

A cell-permeable allosteric, selective inhibitor of Akt1/2 ($IC_{50}$ = 3.5, 42, and 1900 nM against Akt1, Akt2, and Akt3, respectively) that acts in a PH domain-dependent, but not ATP-competitive, manner. Purity: ≥97% by HPLC. M.W. 539.6.

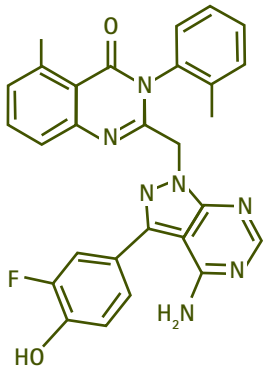



## PI 3-Kδ/γ Inhibitor IX, SW-14

**(2-((4-amino-3-(3-fluoro-4-hydroxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl)-5-methyl-3-o-tolylquinazolin-4(3H)-one)**

**(Qty: 5 mg, Catalogue No. 526560)**

A cell permeable, ATP competitive inhibitor of PI 3-Kδ/γ that displays 70-fold selectivity over PI 3-Kβ and 900-fold selectivity over PI 3-Kα ($IC_{50}$ = 9 nM for PI3Kδ and 21 nM for PI 3-Kγ). Purity: ≥95% by HPLC. M.W. 507.5.

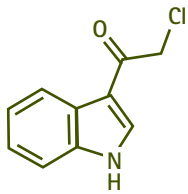



## Akt Inhibitor XIX, 3CAI

**3-Chloroacetyl-indole, 2-Chloro-1-(1H-indol-3-yl)ethanone**

**(Qty: 25 mg, Catalogue No. 124037)**

A cell-permeable chloroacetyl-indole compound that inhibits Akt1 and Akt2 kinase activity ($IC_{50}$ <1 µM) by directly targeting Akt PH domain, exhibiting little or much reduced activity against a panel of 84 other kinases. Effectively inhibits Akt-mediated downstream effector proteins phosphorylation in a time-dependent manner, resulting in effective apoptosis induction in HCT116 and HT29 cultures (by 55% and 60%, respectively; 4 µM for 4 days). Purity: ≥98% by HPLC. M.W. 193.6.




## Akt Inhibitor XVIII, SC66

**(2E,6E)-2,6-bis(4-Pyridylmethylene) cyclohexanone**

**(Qty: 25 mg, Catalogue No. 124036)**

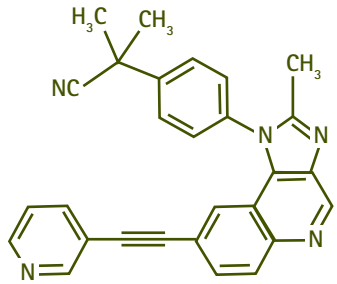
A cell-permeable compound that represses Akt activation by allosterically disrupting Akt-PH domain binding to PIP3 and directly facilitating Akt ubiquitination with little proteasomal or deubiquitination activity. Blocks phosphorylation of Akt-$Ser^{473}$ and downstream targets. Purity: ≥98% by HPLC. M.W. 276.3.

## PI 3-K/PDK-1 Inhibitor, NVP-BAG956

**(α,α,-Dimethyl-4-(2-methyl-8-(2-(3-pyridinyl) ethynyl)-1H-imidazo[4,5-c]quinolin-1-yl)-benzeneacetonitrile)**

**(Qty: 5 mg, Catalogue No. 528121)**

PDK1 is a member of the PI 3-kinase family and is also an activator of Akt. In fact, PI 3-kinase generates PIP3, which acts as a scaffold to bring PDK1 and Akt together. For strong inhibition of PI 3-kinase signaling, use this cell-permeable, potent, reversible and ATP-competitive dual kinase inhibitor of PI 3-K/PDK1 ($IC_{50}$ = 56, 446, 35, 117 and 245 nM for p110α, p110β, p110δ, p110γ and PDK1, respectively; $IC_{50}$ = 45 nM for pAkt-T308 in U87MG cells). Purity: ≥98% by HPLC. M.W. 427.5.

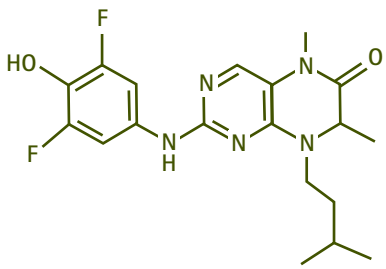


## RSK Inhibitor II

**(2-(3,5-Difluoro-4-hydroxy-anilino)-8-isopentyl-5,7-dimethyl-7H-pteridin-6-one, racemic)**

**(Qty: 5 mg, Catalogue No. 559286)**

A cell-permeable, potent, reversible, and ATP-competitive inhibitor of pan-RSK ($IC_{50}$ = 31, 24, 18 and 15 nM for RSK1, 2, 3 & 4, respectively; [ATP] 100 μM). Purity: ≥95% by HPLC. M.W. 391.4.

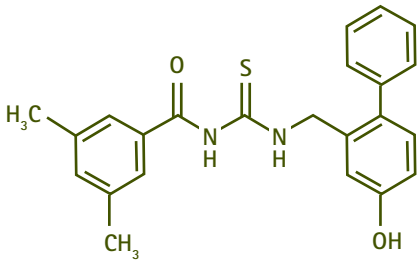


## $PIP_3$ Antagonist, PITenin-7

**(N-(4-Hydroxybiphenyl-3-ylcarbamothioyl)-3,5-dimethylbenzamide)**

**(Qty: 10 mg, Catalogue No. 524618)**

A cell-permeable phosphatidylinositol-3,4,5-triphosphate ($PIP_3$) mimic that inhibits PI 3-K/$PIP_3$/Akt signaling. Shown to disrupt PIP3/Akt1 PH domain interaction over $PIP_3$/PDK1 PH ($IC_{50}$ = 13.4 and 52.3 μM) in a reversible manner. Purity: ≥95% by HPLC. M.W. 376.5.

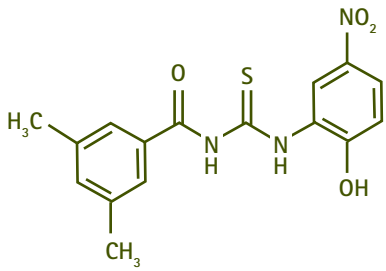


## $PIP_3$ Antagonist II, DM-PIT-1

**[[(2-hydroxy-5-nitrophenyl)amino] thioxomethyl]-3,5-dimethyl-benzamide**

**(Qty: 25 mg, Catalogue No. 524619)**

A cell-permeable benzoylthiourea compound that is shown to compete against $PIP_3$ (Catalogue No. 524615) for binding PH domains of Akt1 ($IC_{50}$ ≥31 μM), ARNO, GRP1, and PKD1. Effectively blocks $PIP_3$-dependent cellular PI3K-PDK1-Akt signaling pathway activation in U87MG (25 to 100 μM for 3 d) and PDGF-induced Akt and GRP membrane translocation in serum-starved SUM159 cells (1 h 100 μM pretreatment), while being inactive against PDGF-induced Btk translocation or PMA-induced PLC-δ and TAPP1/2 translocations. Purity: ≥98% by HPLC. M.W. 345.4.



## PI 3-K/mTOR Inhibitor III, PKI-179

**(Qty: 5 mg, Catalogue No. 526561)**

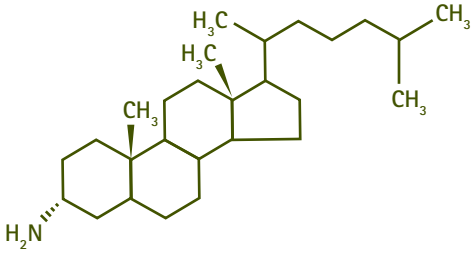
A potent, reversible, and ATP-competitive dual mTOR and PI 3-K inhibitor ($IC_{50}$ =0.42, 8, 24, 74, 77, 14 and 11 nM for mTOR, PI 3-Kα, -β, -γ, -δ, and PI 3-Kα mutants E545K and H1047R, respectively) with excellent selectivity over 361 kinases ($IC_{50}$ >50 µM). Blocks Akt-$Thr^{308}$ and Akt-$Ser^{473}$ phosphorylation, and induces apoptosis and cell death. Purity: ≥98% by HPLC. M.W. 506.6.

## SHIP1 Inhibitor, 3AC

(3α-Aminocholestane)

**(Qty: 10 mg, Catalogue No. 565835)**

A potent ($IC_{50}$ = 10 µM) and specific inhibitor of SHIP1. Inhibits SHIP1-mediated immune response and blocks SHIP1-dependent cancer cell survival. Purity: ≥95% by NMR. M.W. 387.7.

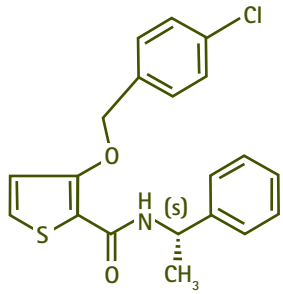



## SHIP2 Inhibitor, AS1938909

3-(2,4-Dichlorobenzyl)oxy]-N-(2,6-difluorobenzyl)thiophene-2-carboxamide

**(Qty: 10 mg, Catalogue No. 565840)**

A cell-permeable thiophenecarboxamide compound that is shown to increase glucose metabolism and activate intracellular insulin signaling. Acts as a potent, competitive and reversible inhibitor of SHIP2 activity (Ki = 0.44 µM for hSHIP2) with moderate to excellent selectivity over SHIP1 and other related phosphatases ($IC_{50}$ = 0.18, 0.57, 21, > 50, > 50 and > 50 µM for mSHIP2, hSHIP2, hSHIP1, hPTEN, h-synaptojanin and h-myotubularin, respectively). Purity: ≥99% by HPLC. M.W. 371.9.




## Convenient, ready-to-use solutions

### InSolution™ Akt Inhibitor V, Triciribine

**(Qty: 2 mg, Catalogue No. 124038)**

2 mg/312 µL solution of Akt Inhibitor V, Triciribine in DMSO. Purity: ≥95% by HPLC.

### InSolution™ RSK Inhibitor, SL0101

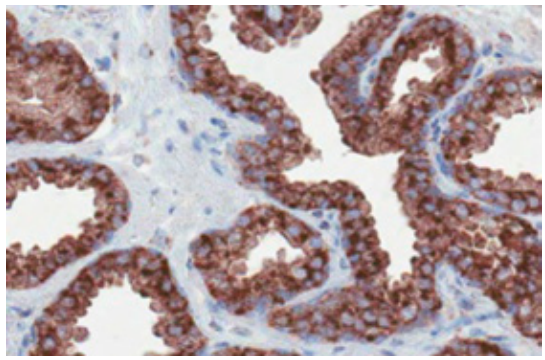
**(Qty: 500 µg, Catalogue No. 559292)**

A 10 mM (500 µg/97 µL) solution of RSK Inhibitor, SL0101 in DMSO. Purity: ≥95% by HPLC.

## Anti-Girdin, C-terminus, clone 10E6.1

**(Catalogue No. MABT100)**

Girdin interacts with actin via its C-terminal domain, and may play a crucial role in cell motility and cytoskeleton remodeling. These effects may be regulated by a reciprocal relationship between girdin and Akt. Akt phosphorylates girdin, and girdin binds to Akt and enhances phosphorylation of Akt. Girdin/Akt signaling may facilitate the proliferation of cancer cells.

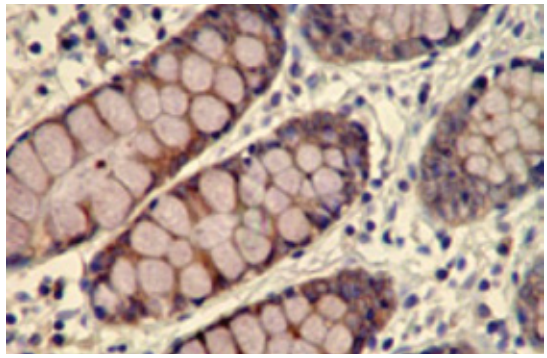


**Immunohistochemistry Analysis:** A 1:250 dilution from a representative lot detected girdin in epithelial cells of human prostate tissue.

## Anti-RSK1, clone E4, Rabbit Monoclonal

**(Catalogue No. 04-417)**

RSK1, like other RSK isoforms, is activated in response to mitogenic stimuli, including extracellular signal-regulated protein kinases Erk1 and Erk2. RSK 1 is activated by MAPK *in vitro* and *in vivo* via phosphorylation. Active RSKs appear to play a major role in transcriptional regulation by translocating to the nucleus and phosphorylating c-Fos and CREB.

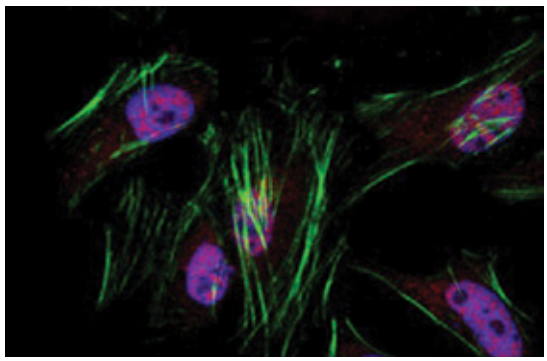


**Immunohistochemistry Analysis:** Colon tissue section stained for RSK1 subcellular location using a representative lot of Anti-RSK1, clone E4 at 1:100 dilution.

## Anti-SKAR

**(Catalogue No. ABS157)**

SKAR (S6K1 Aly/REF-like substrate) is a nuclear protein that is localized at exon junction complexes (EJC) which interact with pre-mRNA molecules to ensure that correct splicing has occurred. SKAR recruits ribosomal protein S6 kinase 1 (S6K1) to EJCs and links S6K1 to the mTOR pathway.



**Immunocytochemistry Analysis:** Confocal fluorescent analysis of HeLa cells using a 1:500 dilution of a representative lot of Anti-SKAR (Red). Actin filaments have been labeled with Alexa Fluor® 488 dye - Phalloidin (Green). Nucleus is stained with DAPI (Blue). This antibody positively stains the nucleus.

## Ordering Information

| Description | Species | Applications | Qty/Pk | Catalogue No. |
|---|---|---|---|---|
| Anti-Girdin, C-terminus, clone 10E6.1 | Human | IC, IH (Paraffin), IP, WB | 100 µL | MABT100 |
| Anti-RSK1, clone E4, Rabbit Monoclonal | H,M,R | FC, IC, IH, IP, WB | 100 µL | 04-417 |
| Anti-SKAR | H,R | IC, IH | 100 µL | ABS157 |

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