Cell lines validated for use with AlphaScreen® *SureFire*® kits

Assay Kit	Upstream Cell Surface Receptors	Cell Line or Cell Type	Activator	Inhibitors	General Information
AlphaScreen® SureFire® Phospho-ERK 1/2 Assay Kit	GPCRs, mitogens, growth factors, cytokines	CHO, HEK 293,			Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular programs such as cell proliferation, differentiation, motility, and death. The p44/42 MAPK (ERK1/2) signaling pathway can be activated in response to a diverse range of extracellular stimuli including mitogens, growth factors, and cytokines and is an important target in the diagnosis and treatment of cancer. Several downstream targets of ERK1/2 have been identified, including p90RSK and the transcription factor Elk-1. ERK1/2 are negatively regulated by a family of dual-specificity (Thr/Tyr) MAPK phosphatases, known as DUSPs or MKPs, along with MEK inhibitors such as U0126 and PD98059.
		Sw 3T3	Betacellulin, Bombesin	MEK inhibitors	
		CHO A1	Adenosine	MEK inhibitors	
		CHO M4	Carbachol	Atropine, U0126, PD98059	
		CHO M1	Carbachol	Atropine, U0126, PD98059	
		CHO BK2	bradykinin	MEK inhibitors	
		NIH 3T3	PDGF	MEK inhibitors	
		HEK 293 V1b	Vasopressin	MEK inhibitors	
		A431	EGF	MEK inhibitors	
		MCF7	EGF	MEK inhibitors	
AlphaScreen® SureFire® Total ERK Assay Kit		A431			
AlphaScreen® SureFire® Phospho-AKT (Ser473) Assay Kit	RTKs, insulin	NIH 3T3	PDGF	PI3K inhibitors	Akt (also known as PKB) plays a critical role in controlling survival and apoptosis. This protein kinase is activated by various growth and survival factors involving the PI3 kinase pathway. Akt is activated by phospholipid binding and activation loop phosphorylation at Thr308 by PDK1. Phosphorylation of Akt at Ser473 is mediated by mammalian target of rapamycin (mTOR) in a rapamycin-insensitive complex with rictor and Sin1. Akt promotes cell survival by phosphorylating and inactivating several pro-apoptotic targets, including Bad, forkhead transcription factors, c-Raf and caspase-9. Another essential Akt function is the regulation of glycogen synthesis through phosphorylation and inactivation of GSK-3α and β. In addition to its role in survival and glycogen synthesis, Akt is involved in cell cycle regulation by preventing GSK-3β mediated phosphorylation and degradation of cyclin D1 and by negatively regulating the cyclin dependent kinase inhibitors p27 Kip and p21 Waf1/CIP1.
		HEK293	serum	PI3K inhibitors	
		PC3	serum	PI3K inhibitors	
		U87	serum	PI3K inhibitors	
		A2780	serum	PI3K inhibitors	
		MDA MB 231	serum	PI3K inhibitors	
		LNCaP	serum	PI3K inhibitors	
		LS513	serum	PI3K inhibitors	
		MCF-7	insulin	PI3K inhibitors	
AlphaScreen® SureFire® Phospho-AKT (Thr308) Assay Kit	RTKs, insulin	NIH 3T3	PDGF	PI3K inhibitors	
		HEK293	serum	wortmannin	
		A2780	serum	PI3K inhibitors	
		LNCaP	serum	PI3K inhibitors	
		LS513	serum	PI3K inhibitors	
		MCF-7	insulin	PI3K inhibitors	
AlphaScreen® SureFire® Phospho-p38 MAPK Assay Kit	osmotic shock, inflammatory cytokines, lipopolysaccharides (LPS), UV light and growth factors	U937	TNFalpha	anti-hTNFa, anti-TNFa R1, soluble TNFa R1	p38 MAP kinase (MAPK) participates in a signaling cascade controlling cellular responses to cytokines and stress. Four isoforms of p38 MAP kinase, p38α, β, γ (also known as ERK6 or SAPK3) and δ (also known as SAPK4) have been identified. Similar to the SAPK/JNK pathway, p38 MAP kinase is activated by a variety of cellular stresses including osmotic shock, inflammatory cytokines, lipopolysaccharides (LPS), UV light and growth factors. MKK3, MKK6 and SEK activate p38 MAP kinase by phosphorylation at Thr180 and Tyr182. Activated p38 MAP kinase has been shown to phosphorylate and activate MAPKAP kinase 2 and to phosphorylate the transcription factors ATF-2, Max and MEF2.
		Hela	TNFalpha		
		RAW 246.7	TGFbeta		
AlphaScreen® SureFire® Phospho-p70 S6K (Thr389) Assay Kit	Akt/mTOR signaling	MCF-7	Insulin	PI3K inhibitors, Rapamycin	p70 S6 kinase is a mitogen activated Ser/Thr protein kinase that is required for cell growth and G1 cell cycle progression. p70 S6 kinase phosphorylates the S6 protein of the 40S ribosomal subunit and is involved in translational control of 5' oligopyrimidine tract mRNAs. A second isoform, p85 S6 kinase, is derived from the same gene and is identical to p70 S6 kinase except for 23 extra residues at the amino terminus, which encode a nuclear localizing signal. Both isoforms lie on a mitogen activated signaling pathway downstream of phosphoinositide-3 kinase (PI-3K) and the target of rapamycin, FRAP/mTOR, a pathway distinct from the Ras/MAP kinase cascade. The activity of p70 S6 kinase is controlled by multiple phosphorylation events located within the catalytic, linker and pseudosubstrate domains. Phosphorylation of Thr229 in the catalytic domain and Thr389 in the linker domain are most critical for kinase function. Phosphorylation of Thr389, however, most closely correlates with p70 kinase activity in vivo. Prior phosphorylation of Thr389 is required for the action of phosphoinositide 3-dependent protein kinase 1 (PDK1) on Thr229. Thr421/Ser424 phosphorylation is MAPK-dependant.
		NIH 3T3	PDGF	PI3K inhibitors, Rapamycin	
		HEK293	serum	PI3K inhibitors, Rapamycin	
		PC3	serum	PI3K inhibitors, Rapamycin	
		A2780	serum	PI3K inhibitors, Rapamycin	
		MDA MB 231	serum	PI3K inhibitors, Rapamycin	
		LNCaP	serum	PI3K inhibitors, Rapamycin	
AlphaScreen® SureFire® Phospho-p70 S6K (Thr421/Ser424) Assay Kit	Akt/mTOR signaling - MAPK mediated site	MCF-7	EGF	MEK inhibitors, PI3K inhibitors	
		NIH 3T3	PDGF	MEK inhibitors, PI3K inhibitors	
		Hela	EGF	MEK inhibitors, PI3K inhibitors	
		HEK293	serum	MEK inhibitors, PI3K inhibitors	
		LNCaP	serum	MEK inhibitors, PI3K inhibitors	
		PC3	serum	MEK inhibitors, PI3K inhibitors	
		A2780	serum	MEK inhibitors, PI3K inhibitors	
AlphaScreen® SureFire® Phospho-p70 S6K (Thr229) Assay Kit	Akt/mTOR signaling	NIH 3T3	PDGF	PI3K inhibitors, Rapamycin	
		MCF-7	Insulin	PI3K inhibitors, Rapamycin	
		Hela	EGF	LY294002	
		HEK293	serum	wortmannin	
		PC3	serum	PI3K inhibitors, Rapamycin	
		A2780	serum	PI3K inhibitors, Rapamycin	
		LNCaP	serum	PI3K inhibitors, Rapamycin	
AlphaScreen® SureFire® Phospho-MEK 1 Assay Kit	GPCRs, mitogens, growth factors, cytokines	NIH 3T3	Serum, PDGF	PD98059	MEK-1, specifically phosphorylates the MAP kinase regulatory threonine and tyrosine residues present in the Thr-Glu-Tyr motif of ERK. A second MEK family member, MEK-2, resembles MEK-1 in its substrate specificity. Phosphorylation on Ser/Thr by MAP kinase kinase kinases (RAFor MEKK1) positively regulates MEK activity.
		A431	EGF	PD98059, EGF receptor inhibitor	
		Jurkats	PMA		
		Hela	EGF	PD98059, EGF receptor inhibitor	

**MEK inhibitors: PD98059, U0126
**PI3K inhibitors: LY294002, wortmannin, UCN-01 (PDK-1 inhibitor), staurosporine (PDK-1 inhibitor)

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Assay Kit	Upstream Cell Surface Receptors	Cell Line or Cell Type	Activator	Inhibitors	General Information
AlphaScreen® SureFire® Phospho-JNK 1/3 Assay Kit	UV and gamma radiation, ceramides, inflammatory cytokines and in some instances, by growth factors and GPCR agonists	HEK 293	Sorbitol, anisomycin		The stress-activated protein kinase/Jun-amino-terminal kinase (SAPK/JNK) is potently and preferentially activated by a variety of environmental stresses, including UV and gamma radiation, ceramides, inflammatory cytokines and in some instances, by growth factors and GPCR agonists.There are three SAPK/JNK genes with further diversification resulting from alternative splicing. Active SAPK/JNK dimers can translocate to the nucleus to regulate transcription through its effects on c-Jun, ATF-2 and other transcription factors.
AlphaScreen® SureFire® Phospho-STAT 3 (Tyr705) Assay Kit	many cytokines and growth-factor receptors	A431	EGF		The Stat3 transcription factor is an important signaling molecule for many cytokines and growth-factor receptors and is required for murine fetal development. Stat3 is constitutively activated in a number of human tumors and possesses oncogenic potential and anti-apoptotic activities. Stat3 is activated by phosphorylation at Tyr705, which induces dimerization, nuclear translocation and DNA binding.
AlphaScreen® SureFire® Phospho-STAT 5 (Tyr694/Tyr699) Assay Kit	a wide variety of ligands including IL-2, GM-CSF, growth hormone and prolactin	A431	EGF	JAK inhibitor	Stat5 is activated in response to a wide variety of ligands including IL-2, GM-CSF, growth hormone and prolactin. Phosphorylation at Tyr694 is obligatory for Stat5 activation. This phosphorylation is mediated by Src upon erythropoietin stimulation. Stat5 has been found to be constitutively active in some leukemic cell types. Phosphorylated Stat5 is found in some endothelial cells treated with IL-3, which suggests its involvement in angiogenesis and cell motility. Stat5α and β are independently regulated and activated in various cell types. For instance, both isoforms are activated in response to IFN-α in B cells, but only Stat5α is phosphorylated in response to IFN-α in HeLa cells.
		TF-1	IL3	JAK inhibitor	
		KIT225	IL2	JAK inhibitor	
		HEL	serum (constitutive)	JAK inhibitor	
		MO7e	? (beta validation)	JAK inhibitor	
		SET2	? (beta validation)	JAK inhibitor	
		CMK	? (beta validation)	JAK inhibitor	
AlphaScreen® SureFire® Phospho-4EBP 1 (Thr70) Assay Kit	Both the PI3 kinase/Akt pathway and FRAP/mTOR kinase regulate 4E-BP1 activity	NIH 3T3	PDGF	Rapamycin	Translation repressor protein 4E-BP1 (also known as PHAS-1) inhibits cap-dependent translation by binding to the eIF4E translation initiation factor. Hyperphosphorylation of 4E-BP1 disrupts this interaction and results in activation of cap-dependent translation. Both the PI3 kinase/Akt pathway and mTOR kinase regulate 4E-BP1 activity. Multiple 4E-BP1 residues are phosphorylated in vivo. While phosphorylation by mTOR on Thr37 and Thr46 does not prevent the binding of 4E-BP1 to eIF4E, it is thought to prime 4E-BP1 for subsequent phosphorylation at Ser65 and Thr70.
		HEK	serum	wortmannin	
		Hela	EGF	LY294002	
AlphaScreen® SureFire® Phospho-4EBP 1 (Thr37/Thr46) Assay Kit		MCF7	insulin	PI3K inhibitors, Rapamycin	
AlphaScreen® SureFire® Phospho-SMAD 2 (Ser465/Ser467) Assay Kit	TGFbeta superfamily signaling	RAW 246.7	TGFbeta		Members of the Smad family of signal transduction molecules are components of a critical intracellular pathway that transmits TGF-β signals from the cell surface into the nucleus. Three distinct classes of Smads have been defined: the receptor-regulated Smads (R-Smads), which include Smad1, 2, 3, 5 and 8, the common-mediator Smad (co-Smad), Smad4, and the antagonistic or inhibitory Smads (I-Smads), Smad6 and 7. Activated type I receptors associate with specific R-Smads and phosphorylate them on a conserved carboxy-terminal SSXS motif. The phosphorylated R-Smad dissociates from the receptor and forms a heteromeric complex with the co-Smad (Smad4), allowing translocation of the complex to the nucleus. Once in the nucleus, Smads can target a variety of DNA binding proteins to regulate transcriptional responses.
		C2C12	TGFbeta		
AlphaScreen® SureFire® Phospho-PDK 1 (Ser241) Assay Kit	Constitutive	MCF7	constitutive	Staurosporine, UCN-01	Phosphoinositide-dependent protein kinase 1 (PDK1) plays a central role in many signal transduction pathways, activating Akt and the PKC isoenzymes p70 S6 kinase and RSK. Through its effects on these kinases, PDK1 is involved in the regulation of a wide variety of processes, including cell proliferation, differentiation and apoptosis.
AlphaScreen® SureFire® Phospho-GSK 3b (Ser9) Assay Kit	Insulin, Akt signaling	PC3	serum	PI3K inhibitors	Glycogen synthase kinase-3 (GSK-3) was initially identified as an enzyme that regulated glycogen synthesis in response to insulin. GSK-3 is involved in a diverse array of signaling pathways, including glycogen synthesis and cellular adhesion, and has been implicated in Alzheimer's disease. Tau, a microtubule-binding protein which serves to stabilize microtubules in growing axons, is found to be hyper-phosphorylated in paired helical filaments (PHF), the major fibrous component of neurofibrillary lesions associated with Alzheimer's disease. Hyperphosphorylation of Tau is
		LNCaP	serum	PI3K inhibitors	
		A2780	serum	PI3K inhibitors	
		MCF-7	insulin	PI3K inhibitors	
AlphaScreen® SureFire® Phospho-GSK 3a (Ser21) Assay Kit	Insulin, Akt signaling	PC3	Insulin	PI3K inhibitors	
		MCF-7	Insulin	PI3K inhibitors	
		A2780	serum	PI3K inhibitors	
		LNCaP	serum	PI3K inhibitors	

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Assay Kit	Upstream Cell Surface Receptors	Cell Line or Cell Type	Activator	Inhibitors	General Information
AlphaScreen® SureFire® Phospho-BAD (Ser112) Assay Kit	Survival factors (i.e. growth factors, cytokines)	PC3	serum	PI3K inhbitors	Bad is a proapoptotic member of the Bcl-2 family that promotes cell death by displacing Bax from binding to Bcl-2 and Bcl-xL. Survival factors (i.e. growth factors, cytokines) inhibit the apoptotic activity of Bad by activating intracellular signaling pathways that result in the phosphorylation of Bad at Ser112 and Ser136. Phosphorylation at these sites promotes binding of Bad to 14-3-3 protein to prevent an association between Bad and Bcl-2 and Bcl-xl. Akt phosphorylates Bad at Ser136, and at Ser112 both in vivo and in vitro by p90RSK and mitochondria-anchored PKA.
		LNCaP	serum	PI3K inhbitors	
		MCF-7	insulin	PI3K inhbitors	
		A2780	serum	PI3K inhbitors	
		MDA MB 231	serum	PI3K inhbitors	
AlphaScreen® SureFire® Phospho-BAD (Ser136) Assay Kit		MCF7	insulin	PI3K inhibitors	
AlphaScreen® SureFire® Phospho-S6 RP (Ser235/Ser236) Assay Kit	growth factors	NIH 3T3	PDGF	PI3K inhibitors, rapamycin	One way that growth factors and mitogens effectively promote sustained cell growth and proliferation is by upregulating mRNA translation. Growth factors and mitogens induce the activation of p70 S6 kinase and the subsequent phosphorylation of the S6 ribosomal protein. Phosphorylation of S6 ribosomal protein correlates with an increase in translation of mRNA transcripts that contain an oligopyrimidine tract in their 5' untranslated regions. These particular mRNA transcripts (5'TOP) encode proteins involved in cell cycle progression as well as ribosomal proteins and elongation factors necessary for translation. Important S6 ribosomal protein phosphorylation sites include several residues (Ser235, Ser236, Ser240 and Ser244) located within a small, carboxy-terminal region of the S6 protein.
		A2780	serum	PI3K inhibitors, rapamycin	
		LNCaP	serum	PI3K inhibitors, rapamycin	
		LS513	serum	PI3K inhibitors, rapamycin	
		MDA MB 231	serum	PI3K inhibitors, rapamycin	
		U87	serum	PI3K inhibitors, rapamycin	
		PC3	serum	PI3K inhibitors, rapamycin	
AlphaScreen® SureFire® Phospho-S6 RP (Ser240/Ser244) Assay Kit	growth factors	NIH 3T3	PDGF	PI3K inhibitors, rapamycin	
		A2780	serum	PI3K inhibitors, rapamycin	
		LNCaP	serum	PI3K inhibitors, rapamycin	
		LS513	serum	PI3K inhibitors, rapamycin	
		MDA MB 231	serum	PI3K inhibitors, rapamycin	
		U87	serum	PI3K inhibitors, rapamycin	
		PC3	serum	PI3K inhibitors, rapamycin	
AlphaScreen® *SureFire*® p-IkB (Ser32/Ser36)	inflammatory cytokines, growth factors and chemokines	Hela	TNFalpha		The NF-κB/Rel transcription factors are present in the cytosol in an inactive state complexed with the inhibitory IκB proteins. Activation occurs via phosphorylation of IκB-α at Ser32 and Ser36 followed by proteasome-mediated degradation that results in the release and nuclear translocation of active NF-κB. IκB-α phosphorylation and resulting Rel-dependent transcription are activated by a highly diverse group of extracellular signals including inflammatory cytokines, growth factors and chemokines. Kinases that phosphorylate IκB at these activating sites have been identified. Because phosphorylation of IκB-α at Ser32/36 is essential for release of active NF-κB, phosphorylation at this site is an excellent marker of NF-κB activation.
AlphaScreen® *SureFire*® p-IKKalpha (Ser176/Ser180)	inflammatory cytokines, growth factors and chemokines	Hela	TNFalpha		The NF-κB/Rel transcription factors are present in the cytosol in an inactive state, complexed with the inhibitory IκB proteins. Most agents that activate NF-κB do so through a common pathway based on phosphorylation-induced, proteasome-mediated degradation of IκB. The key regulatory step in this pathway involves activation of a high molecular weight IκB kinase (IKK) complex, whose catalysis is generally carried out by three tightly associated IKK subunits. IKKα and IKKβ serve as the catalytic subunits of the kinase. IKKγ serves as the regulatory subunit. Activation of IKK depends on phosphorylation; Ser177 and Ser181 in the activation loop of IKKβ (176 and 180 in IKKα) are the specific sites whose phosphorylation causes conformational changes resulting in kinase activation.
AlphaScreen® *SureFire*® p-IKKbeta (Ser177/Ser181)	inflammatory cytokines, growth factors and chemokines	Hela	TNFalpha		
AlphaScreen® *SureFire*® p-NFkB (Ser536)	inflammatory cytokines, growth factors and chemokines	Hela	TNFalpha		Transcription factors of the nuclear factor κB (NF-κB)/Rel family play a pivotal role in inflammatory and immune responses. There are five family members in mammals: RelA (p65), c-Rel, RelB, NF-κB1 (p105/p50) and NF-κB2 (p100/p52). Both p105 and p100 are proteolytically processed by the proteasome to produce p50 and p52, respectively. The p50 and p52 products form dimeric complexes with Rel proteins, which are then able to bind DNA and regulate transcription. The most abundant form in most cell types is the p65 (RelA):p50 complex. In unstimulated cells, NF-κB is sequestered in the cytoplasm by its inhibitory proteins, the IκBs. NF-κB-activating agents can induce the phosphorylation of IκBs, targeting them for rapid degradation through an ubiquitin-proteasome pathway, releasing NF-κB to enter the nucleus, where it regulates gene expression.

**MEK inhibitors: PD98059, U0126
**PI3K inhibitors: LY294002, wortmannin, UCN-01 (PDK-1 inhibitor), staurosporine (PDK-1 inhibitor)

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Assay Kit	Upstream Cell Surface Receptors	Cell Line or Cell Type	Activator	Inhibitors	General Information
AlphaScreen® *SureFire*® p-mTOR (Ser2481)	nutrients, PI3K	Hela, PC3	serum	PI3K inhibitors	The mammalian target of rapamycin (mTOR, FRAP, RAFT) is a Ser/Thr protein kinase that functions as an ATP and amino acid sensor to balance nutrient availability and cell growth. When sufficient nutrients are available, mTOR responds to a phosphatidic acid-mediated signal to transmit a positive signal to p70 S6 kinase and participate in the inactivation of the eIF4E inhibitor, 4E-BP1. These events result in the translation of specific mRNA subpopulations. mTOR is phosphorylated at Ser2448 via the PI3 kinase/Akt signaling pathway and autophosphorylated at Ser2481. mTOR plays a key role in cell growth and homeostasis and may be abnormally regulated in tumors. For these reasons, mTOR is currently under investigation as a potential target for anti-cancer therapy.
AlphaScreen® *SureFire*® p-Caspase9 (Ser196)		Hela, LNCaP, PC3	serum	Staurosporine	Ced-3/caspase-1 family members function as key components of the apoptotic machinery and act to destroy specific target proteins which are critical to cellular longevity. Caspase-3, caspase-7 and caspase-9, but not caspase-1, have been shown to cleave the 112 kDa nuclear protein PARP into an 85 kDa apoptotic fragment. Caspase-9 can be directly regulated by protein phosphorylation. Akt phosphorylates caspase-9 in vitro on serine 196 and inhibits its protease activity.
AlphaScreen® *SureFire*® p-EGF Receptor (Tyr1068)	n/a	A431	EGF, other EGFR agonists	AG1478	Epidermal growth factor receptor (EGFR) is a tyrosine kinase receptor belonging to the HER/ErbB family. Ligand binding results in dimerization, autophosphorylation, and activation of downstream signaling pathways, regulated via multiple phosphorylation events. The GRB2 adaptor protein binds activated EGFR at phospho-Tyr1068. GRB2 forms a complex with activated EGFR and the Ras-specific guanine nucleotide exchange factor Sos 1, and, together, they regulate the EGFR ligand-mediated activation of Ras.
AlphaScreen® *SureFire*® p-IGF-1 Receptor (Tyr1135/1136)	n/a	A431	IGF-1, Insulin		Insulin-like growth factor receptor 1 (IGF-1R) is a receptor tyrosine kinase that is widely expressed in many different cell types. IGF-1R stimulates growth, blocks apoptosis, and has been implicated in the growth of some types of cancer. Receptor autophosphorylation follows binding of ligands, and phosphorylation of Tyr1131, Tyr1135 and Tyr1136 are among the earliest autophosphorylation events. Phosphorylation of these three tyrosine sites is necessary for receptor activation. Activated IGF-IR associates with Shc, GRB2 and Sos 1, which initiates Ras and ERK kinase cascades, and transcription factor activity.
AlphaScreen® *SureFire*® p-Insulin Receptor (Tyr1150/1151)	n/a	Hela	Insulin		The insulin receptor is a disulfide-linked heterotetrameric complex, consisting of 2 extracellular a subunits and 2 intracellular β subunits. Insulin binding activates the insulin receptor by inducing phosphorylation of tyrosine kinase domain on the b-subunit and recruitment of SH2 and SH3 domain-containing proteins that act as signaling intermediates. Tyrosine autophosphorylation of insulin receptor is one of the earliest cellular responses to insulin stimulation, and full kinase activation requires Tyr1146, Tyr1150 and Tyr1151 phosphorylation. The insulin receptor initiates intracellular signaling pathways that promote glucose uptake and glycogen synthesis. Type 1 diabetes is an auto-immune condition that results in destruction of insulin secreting cells and a loss in insulin-sensitive glucose uptake. Type 2 diabetes is a condition where cells become resistant to insulin.
AlphaScreen® *SureFire*® p-mTOR (Ser2448)	nutrients, PI3K	Hela	Serum, insulin	Rapamycin	The mammalian target of rapamycin (mTOR, FRAP, RAFT) is a Ser/Thr protein kinase that functions as an ATP and amino acid sensor to balance nutrient availability and cell growth. When sufficient nutrients are available, mTOR responds to a phosphatidic acid-mediated signal to transmit a positive signal to p70 S6 kinase and participate in the inactivation of the eIF4E inhibitor, 4E-BP1. These events result in the translation of specific mRNA subpopulations. mTOR is phosphorylated at Ser2448 via the PI3 kinase/Akt signaling pathway and autophosphorylated at Ser2481. mTOR plays a key role in cell growth and homeostasis and may be abnormally regulated in tumors. For these reasons, mTOR is currently under investigation as a potential target for anti-cancer therapy.

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AlphaScreen® *SureFire*® p-ALK (Tyr1586)	n/a	Karpass-299	Serum		Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor belonging to the insulin receptor superfamily. The mechanism of activation of ALK is complex, and appears to involve the pleiotrophin (PTN)/receptor protein-tyrosine phosphatase b/z pathway. In ALK-expressing cells, PTN induces phosphorylation of ALK, and several downstream effectors. ALK is normally expressed specifically in the nervous system. A truncated form containing the catalytic domain of ALK fused to the N-terminal domain of nucleophosmin (NPM-ALK) is expressed as the result of a translocation, and occurs in many non-Hodgkin's lymphomas.
AlphaScreen® *SureFire*® p-ALK (Tyr1604)	n/a	Karpass-299	Serum		Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor belonging to the insulin receptor superfamily. The mechanism of activation of ALK is complex, and appears to involve the pleiotrophin (PTN)/receptor protein-tyrosine phosphatase b/z pathway. In ALK-expressing cells, PTN induces phosphorylation of ALK, and several downstream effectors. ALK is normally expressed specifically in the nervous system. A truncated form containing the catalytic domain of ALK fused to the N-terminal domain of nucleophosmin (NPM-ALK) is expressed as the result of a translocation, and occurs in many non-Hodgkin's lymphomas.
AlphaScreen® *SureFire*® p-Elk1 (Ser383)	MAPK pathway activators	Hek293, HeLa, A431	Serum, PMA		Elk-1 is a transcription factor of the Ets family of DNA-binding proteins, and is a component of a ternary complex that binds the serum response element to control growth factor-mediated gene activity. Elk-1 is phosphorylated at a cluster of Serine/Threonine sites. Phosphorylation of Ser383 in particular, is critical for ELK-1 transcriptional activation activity. Elk-1 appears to be a direct target of ERK 1/2, while further evidence suggests that Elk-1 can be phosphorylated by SAPK/JNK at Ser383.
AlphaScreen® *SureFire*® p-Chk1 (p-Ser345)	DNA damage	Hek293, HeLa, NIH3T3, MCF7	calyculin A	Nocodazol	Chk1 plays an essential role in the mammalian DNA damage checkpoint, embryonic development, and tumor suppression, acting downstream of ATM/ATR kinase. Activation of Chk1 involves phosphorylation of Ser345 in response to blocked DNA replication and particular forms of stress. Activated Chk1 can phosphorylate and inactivate cdc25C, blocking the activation of cdc2 and transition into mitosis.
AlphaScreen® *SureFire*® p-ErbB2 (Tyr1221/1222)	EGFR transactivation	A431	EGF	AG1478 (EGF receptor inhibitor)	ErbB2 is a 185 kDa transmembrane, receptor-like protein with tyrosine kinase activity. ErbB2 lacks an identified ligand, however ErbB2 kinase activity can be activated through associations with other ErbB family members, such as the EGF receptor. Overexpression of ErbB2 is detected in almost 40% of human breast cancers, making ErbB2 a therapeutic target in the treatment of breast cancer. Tyr1221/1222 is a major autophosphorylation site in ErbB2, and phosphorylation couples ErbB2 to the Ras-Raf-MAP kinase signal transduction pathway.
AlphaScreen® *SureFire*® p-c-Jun (Ser63)	JNK pathway activators	NIH3T3, Hek293	serum, anisomycin		c-Jun, a component of the transcription factor AP-1, has transcriptional activity that is regulated by phosphorylation at Ser63 and Ser73. Many factors, including mitogenic and stress-induced signaling pathways, stimulate phosphorylation of c-Jun at Ser63/73 and activate c-Jun-dependent transcription. JNK, whose activity is stimulated by the same signals, binds to the amino-terminal region of c-Jun and phosphorylates it at Ser63/73.
AlphaScreen® *SureFire*® p-c-Jun (Ser73)	JNK pathway activators	NIH3T3, Hek293	serum, anisomycin		c-Jun, a component of the transcription factor AP-1, has transcriptional activity that is regulated by phosphorylation at Ser63 and Ser73. Many factors, including mitogenic and stress-induced signaling pathways, stimulate phosphorylation of c-Jun at Ser63/73 and activate c-Jun-dependent transcription. JNK, whose activity is stimulated by the same signals, binds to the amino-terminal region of c-Jun and phosphorylates it at Ser63/73.

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