# **CELL SIGNALING ASSAY KITS**



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## **Ordering**

#### ONLINE:

www.ArborAssays.com/order-form

Inhibitors and Activators

### PHONE:

Call 734-677-1774 or Toll Free: 855-677-1774. Monday-Friday 8:30 am to 5:30 pm, EST.

## FAX:

Send faxes to 734-677-6860.

## E-MAIL:

Send E-mail orders to Orders@ArborAssays.com



### **DISTRIBUTORS:**

Check our website at <a href="https://www.ArborAssays.com/distributors">www.ArborAssays.com/distributors</a> for a list of distributors.

## 2',3'-Cyclic GAMP ELISA Kits

K067-H1 (1 Strip-Well Plate) | K067-H5 (5 Strip-Well Plate) K067-H1W (1 Whole Plate) | K067-H5W (5 Whole Plate) K067-H1D (384-Well Plate)

#### **FEATURES**

▶ Use Measure 2′,3′-cGAMP in tissues and cells

Sample Cell Lysates, Tissue Extracts, TCM

Sensitivity 0.04 pmol/mL, 2 fmol/well

► Samples/Kit 96-well: 39 or 231 in Duplicate

384-well: 183 in Duplicate

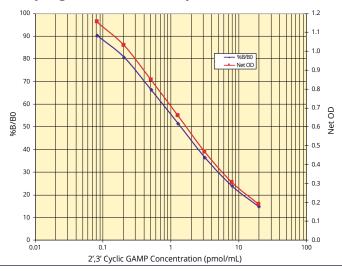
► Stability Liquid 4°C Stable Reagents

Readout Colorimetric, 450 nm



#### SCIENTIFIC RELEVANCE

2',3'-Cyclic guanosine monophosphate-adenosine monophosphate (cyclic GMP-AMP, cGAMP, cyclic [G(2',5') pA(3',5')p]) is referred to as "noncanonical" cGAMP due to the presence of the atypical 2'-5' phosphodiester linkage between the guanosine and the adenosine. 2',3'-Cyclic GAMP is a novel second messenger in innate immunity that regulates type I interferon (IFN) production. Produced in mammalian cells by cGAS (cGAMP synthase) in response to double-stranded DNA in the cytoplasm binding to cGAS, cGAMP binds to the stimulator of interferon genes (STING). Subsequently STING induces the TBK1-IRF3-dependent production of IFN- $\beta$ . This cGAS-cGAMP-STING pathway has been shown to play a critical role in pathogen detection and physiological conditions such as metabolic dysregulation, autoimmunity, and cancer.





## 2',3'-Cyclic GAMP STING-Based FRET Detection Kits

K081-F1 (1 Plate) | K081-H5 (5 Plate)

#### **FEATURES**

Use Detection of 2',3'-cGAMP

Sample Cell Lysates, Tissue Extracts, TCM

Sensitivity 82.02 pmol/mL

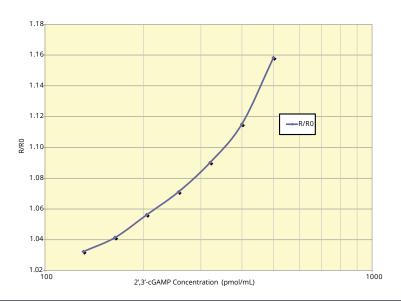
Standard Range 500 - 131 nM

Samples/Kit 40 or 232 in Duplicate

Readout Fluorescence: Excitation 458 nm, Emission: 600 nm/490 nm

### SCIENTIFIC RELEVANCE

The assay uses a biosensor (BioSTING), a fluorescence resonance energy transfer (FRET) based sensor based on STING, designed specifically to detect 2',3'-cGAMP in real-time within living cells. FRET is a fluorescence detection platform based on a distance-dependent relationship between two fluorophores. In the case of BioSTING, mTFP and mKO2 are the fluorophores of choice. When mTFP is excited with a wavelength of 458 nm, the emission is detected at 490 nm when no ligand (2',3'-cGAMP) is present. When BioSTING binds to the ligand, the two fluorophores are positioned in closer proximity to each other, allowing the fluorescence to be transferred from mTFP to mKO2, changing the emission wavelength to 600 nm. Using this detection method, BioSTING shows applicability for in vitro high-throughput screening for cylic di-nucleotide production modulation and direct screening for STING agonists and antagonists.





## 3',3'-Cyclic GAMP ELISA Kits

K073-H1 (1 Plate) | K073-H5 (5 Plate)

#### **FEATURES**

► Use Measure 3′,3′-cGAMP in tissues and cells

Sample Cell Lysates, Tissue Extracts, TCM

► Sensitivity 0.944 pmol/mL, 47 fmol/well

Samples/Kit 39 or 231 in Duplicate

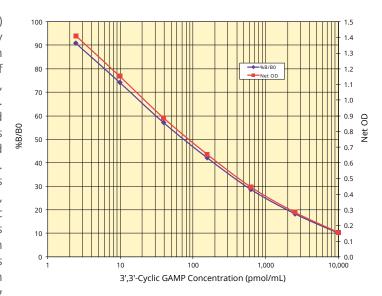
► Stability Liquid 4°C Stable Reagents

Readout Colorimetric, 450 nm



#### SCIENTIFIC RELEVANCE

3',3'-cyclic GAMP (cyclic [G(3',5')pA(3',5') p], previously known as cGAMP) is a key mediator of bacterial signal transduction and regulation, controlling a range of diverse targets including transcription, enzyme activity and cell cycle progression. 3',3'-cGAMP signaling in bacteria is regulated in-part by gene regulatory RNA elements called riboswitches that bind and respond to cGAMP with high affinity and specificity. The 3',3'-cGAMP riboswitches regulate genes involved in motility, biofilm formation, colonization, and virulence. The cyclic nucleotides have emerged as key players involved in bacterial physiology and inhibition studies of cGAMP signaling are ongoing as an anti-microbial strategy. In mammalian cells, 3',3'-cGAMP and its eukaryotic analog



2',3'-cGAMP produced by cGAS, bind STING (stimulator of IFN genes) and subsequently induce TBK1-IRF3-dependent production of IFN- $\beta$ . Here, the cGAS-cGAMP-STING DNA sensing pathway is a key activator of the innate immune response to foreign or harmful native DNA. The cGAS-cGAMP-STING pathway plays a critical role in antiviral and antitumor immunity as well as mediating autoimmune responses. Dysregulation or aberrant activation of the pathway by self-DNA has emerged as an underlying cause of tumorigenesis and autoimmune disorders.



## **Adrenocorticotropic Hormone (ACTH) ELISA Kits**

K072-H1 (1 Plate) | K072-H5 (5 Plate)

#### **FEATURES**

▶ Use Measure ACTH in plasma samples

Sample Plasma

Sensitivity 12.71 pg/mL

Samples/Kit 40 or 232 in Duplicate

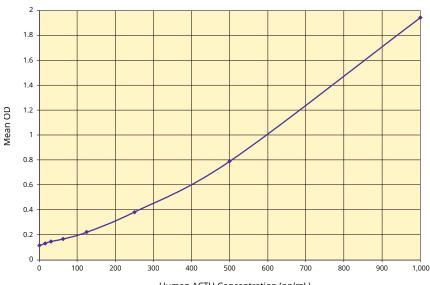
Stability Liquid 4°C Stable Reagents

Readout Colorimetric, 450 nm



#### SCIENTIFIC RELEVANCE

Adrenocorticotropic hormone (ACTH, also adrenocorticotropin, corticotropin) is an important component of the hypothalamic-pituitary-adrenal (HPA) axis and is produced in response to biological stress. Its principal effects are increased production and release of glucocorticoids (GCs). Stress-induced secretion of the peptide hormone corticotropin-releasing hormone (CRH) stimulates pituitary ACTH secretion. Circulating ACTH binds to melanocortin receptors on the surface of adrenal zona cells, inducing the synthesis and release of all adrenal steroids, aldosterone, GCs and adrenal androgens. ACTH is also the principal modulator of cortisol and corticosterone. In addition to the stress response, ACTH synthesis is related to the circadian rhythm in many organisms. Measurement of plasma ACTH is helpful in the differential diagnosis of pituitary Cushing's disease, Addison's disease, adrenal tumors, adrenal hyperplasia, and ectopic ACTH syndrome.





Human ACTH Concentration (pg/mL)



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## **Alkaline Phosphatase Colorimetric Activity Kit**

K082-H1 (1 Plate)

#### **FEATURES**

Use Measure Alkaline Phosphatase in a variety of samples

Sample Serum, non-EDTA plasma, and other biological samples

► Time to answer: 30 minutes end-point assay

Standard Range: 1.563 – 100 mU/mL

Samples/Kit Up to 88 samples in duplicate

Sensitivity 0.06 mU/mL

Stability 4°C liquid reagents

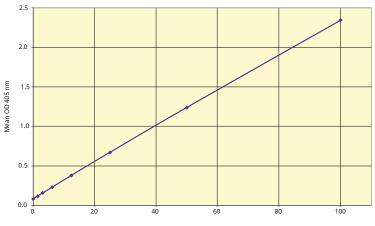
Readout Colorimetric, 405 nm

#### SCIENTIFIC RELEVANCE

Found in many higher organisms, Alkaline Phosphatase (ALP) plays an active role in regulating many biological processes, ranging from metabolism, signal transduction, molecule transportation, and the expression of genetic information. The measurement of ALP activity aids in the study of physiological conditions, disease states, mostly involving the skeletal system and liver, and the structure-activity relationships in inhibitor research.

The DetectX® Alkaline Phosphatase Colorimetric Activity Kit is designed to quantitatively measure ALP activity in a variety of biological samples. The assay is formulated to measure ALP activity in physiologically relevant samples and includes a calibrated ALP standard.

Assay Kit developed by 21 Grams Assays, Inc., www.21gramsassays.com.



Alkaline Phosphatase Activity (mU/mL)



## Cyclic AMP Direct ELISA & Chemiluminescent ELISA Kits

ELISA: K019-H1 (1 Plate) | K019-H5 (5 Plate)

Chemiluminescent ELISA: K019-C1 (1 Plate) | K019-C5 (5 Plate)

#### **FEATURES**

▶ Use Measure cAMP <u>Directly</u>

Sample Cells, Saliva, Urine, Plasma ,Tissue

Convenient Lyse, Stabilize and Measure in One Step

► Sensitivity ELISA: 4.2 fmol /well

CLIA: 0.75 fmol/well

Samples/Kit ELISA: 39 or 231 in Duplicate

CLIA: 38 or 230 in Duplicate

Time to Answer ELISA: 2.5 Hours

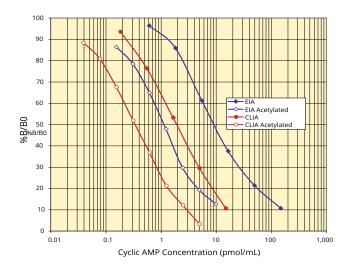
CLIA: 2 Hours

Readout ELISA: Colorimetric, 450 nm

CLIA: Glow Luminescent

### SCIENTIFIC RELEVANCE

Adenosine-3′, 5′-cyclic monophosphate, or cyclic AMP (cAMP), is one of the most important second messengers and a key intracellular regulator. It was discovered by Sutherland and Rall in 1957. Cyclic AMP functions as a mediator of activity for a number of hormones, including epinephrine, glucagon, and ACTH. Adenylate cyclase is activated by the hormones glucagon and adrenaline, and by G protein. Liver adenylate cyclase responds more strongly to glucagon and muscle adenylate cyclase responds more strongly to adrenaline. cAMP decomposition into AMP is catalyzed by the enzyme phosphodiesterase. The Human Metabolome Database lists 166 metabolic enzymes that convert cAMP.



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## Cyclic GMP Direct ELISA & Chemiluminescent ELISA Kits

ELISA: K020-H1 (1 Plate) | K020-H5 (5 Plate)

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Chemiluminescent ELISA: K020-C1 (1 Plate) | K020-C5 (5 Plate)

#### **FEATURES**

▶ Use Measure cGMP Directly

Sample Cells, Saliva, Urine, Plasma, TCM

Convenient Lyse, Stabilize and Measure in One Step

Sensitivity ELISA: 0.188 pmol/mL

CLIA: 1.15 fmol/well

Samples/Kit 39 or 231 in Duplicate

Species Species Independent

► Stability Liquid 4°C Stable Reagents

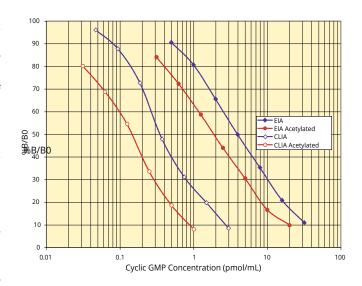
Readout ELISA: Colorimetric, 450 nm

CLIA: Glow Luminescent



#### SCIENTIFIC RELEVANCE

Guanosine 3', 5'-cyclic monophosphate (cyclic GMP; cGMP) is a critical and multifunctional second messenger present at levels typically 10-100 fold lower than cAMP in most tissues. Intracellular cGMP is formed by the action of the enzyme guanylate cyclase (GC) on GTP and degraded through phosphodiesterase hydrolysis. Guanylate cyclases are either soluble or membrane bound. Soluble GCs are nitric oxide responsive, whereas membrane bound GCs respond to hormones such as acetylcholine, insulin and oxytocin. Other chemicals like serotonin and histamine also cause an increase in cGMP levels. Cyclic GMP regulates cellular composition through cGMP-dependent kinase, cGMP-dependent ion channels or transporters, and through its hydrolytic degradation by phosphodiesterase.





## Cyclic GMP Direct ELISA Kits - Improved

K065-H1 (1 Plate) | K065-H5 (5 Plate)

#### **FEATURES**

▶ Use Measure cGMP Directly

Sample Cell and Tissue Lysates, Urine, Plasma, Saliva, TCM

Convenient Lyse, Stabilize and Measure in One Step

Sensitivity 0.091 pmol/mL, 4.55 fmol/well

Samples/Kit 38 or 230 in Duplicate

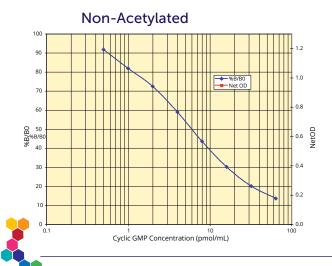
Time to Answer Results in 2.5 Hours

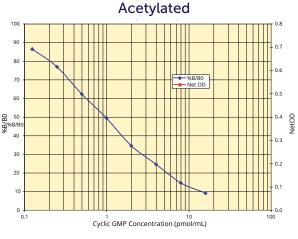
Readout Colorimetric, 450 nm

Comparison Improved Sensitivity and Enhanced Signal over K020-H



Guanosine 3', 5'-cyclic monophosphate (cyclic GMP; cGMP) is a critical and multifunctional second messenger present at levels typically 10-100 fold lower than cAMP in most tissues. Intracellular cGMP is formed by the action of the enzyme guanylate cyclase (GC) on GTP and degraded through phosphodiesterase hydrolysis. Guanylate cyclases are either soluble or membrane bound. Soluble GCs are nitric oxide responsive, whereas membrane bound GCs respond to hormones such as acetylcholine, insulin and oxytocin. Other chemicals like serotonin and histamine also cause an increase in cGMP levels. Cyclic GMP regulates cellular composition through cGMP-dependent kinase, cGMP-dependent ion channels or transporters, and through its hydrolytic degradation by phosphodiesterase.





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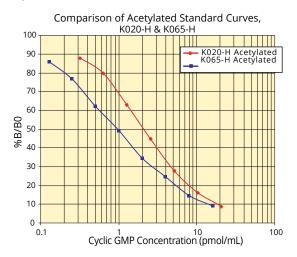
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## Comparison of cGMP ELISA Kits (K065-H vs. K020-H)

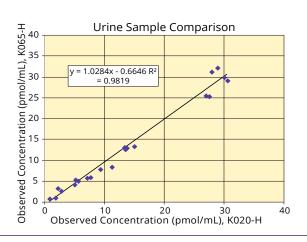
#### ADDITIONAL INFORMATION

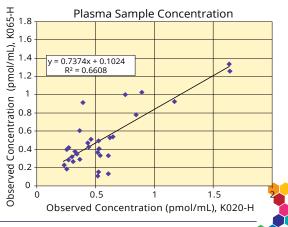
Cyclic GMP levels in most systems are an order of magnitude lower than those found for cyclic AMP and in our constant effort to deliver the easiest, most reliable and sensitive assays we have developed a new cGMP antibody to give you enhanced sensitivity. Our new Cyclic GMP ELISA Kits (K065-H) are 2-fold more sensitive than our current cGMP assay (K020-H).



The improvement in sensitivity is shown by the Limits of Detection and calculated Sensitivity for the 2 assays, with K065-H being half that of K020-H.

Urine dilutions were run in the nonacetylated format of K065-H and K020-H, side by side, and measured cGMP concentrations were similar. Plasma dilutions were run in the acetylated format for each kit with only a slightly larger difference seen between the measured concentrations.





## **Endothelin-1 (ET-1) ELISA Kit**

K045-H1 (1 Plate)

#### **FEATURES**

Use Simplified ET-1 Measurement

Sample Serum, Plasma, TCM

Sensitivity Measures < 0.58 pg/mL ET-1</p>

Economical No C18 Cartridges - Supplied Extraction Solution

Species Species Independent

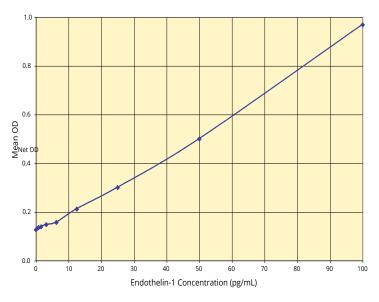
Samples/Kit 39 in Duplicate

Stability Liquid 4°C Stable Reagents

Readout Colorimetric, 450 nm

#### SCIENTIFIC RELEVANCE

Endothelin-1 (ET-1), a peptide of 21 amino acid residues, is a pleiotropic molecule known for its action as a potent vasoconstrictor. ET-1 is one of a family of three proteins encoded by distinct genes. All members of the Endothelin family contain two essential disulfide bridges and six conserved amino acid residues at the C-terminus. Human ET-1 is initially synthesized as a pre-pro-polypeptide of 212 amino acids. It is proteolytically cleaved by a signal peptidase to produce pro-ET-1 and further processed by a Furin-like protease to yield Big ET-1. The vascular endothelium is an abundant source of ET-1. It may also be expressed by leukocytes, smooth muscle cells, mesangial cells, cardiac myocytes, and astrocytes. ET-1 can be induced in endothelial cells by many factors including mechanical



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stimulation, various hormones, and pro-inflammatory cytokines. Production is inhibited by nitric oxide (NO), cyclic nucleotides, prostacyclin, and atrial natriuretic peptide (ANP).



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## **Nitric Oxide Colorimetric Detection Kit**

K023-H1 (2 Plate)

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#### **FEATURES**

▶ Use Measure Nitrite & Nitrate

Sample Water, Serum, Plasma, Urine, Saliva, Lysates, Buffers, TCM

Accurate Calibrated to NIST Standard Reference Material #3185

Sensitivity Highest Optical Density of Any Kit

► Time to Answer 5 Minute Nitrite – 25 Minute Total NO

Samples/Kit 88 in Duplicate

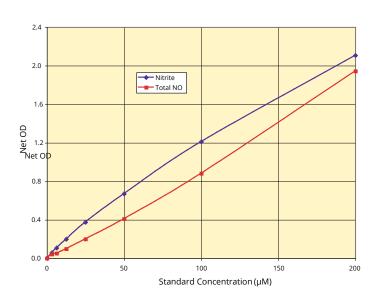
Stability Non-Toxic, Liquid 4°C Stable Reagents

Readout Colorimetric, 550-570 nm

#### SCIENTIFIC RELEVANCE

Nitric oxide (NO) is a diffusible, transient, reactive molecule that has physiological effects in the pM-µM range. Acting through guanylate cyclase activation, NO is an important regulator of the cardiovascular, nervous, and immunological systems. NO is bio-available by two routes. It can be endogenously generated by constitutive or induced NOS enzymes, or it can be ingested as nitrates or nitrites for conversion into NO. The reactive nature of nitric oxide allows it to act as a cytotoxic factor when released during an immune response by macrophages. The reactivity also allows NO to be easily converted to a toxic radical that can produce nitrosylation damage to cells and DNA. Nitrosylation can be a regulated post-translational modification in cell

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signaling. The dynamics of the regulatory/damage facets of NO are major forces in mitochondrial signaling and dysfunction. NO is linked not only to coronary heart disease, endothelial dysfunctions, erectile dysfunction, and neurological disorders, but also diabetes, chronic periodontitis, autism and cancer.



## Prostaglandin E2 (PGE2) Multi-Format ELISA Kits

K051-H1 (1 Strip-Well Plate) | K051-H5 (5 Strip-Well Plate) K051-H1W (1 Whole Plate) | K051-H5W (5 Whole Plate)

#### **FEATURES**

▶ Use Measure in Cells, Saliva, Urine, Serum, Plasma, Tissue

Multi-Format 3 Ranges: 1,000-15.6; 500-39 or 500-1.95 pg/mL

► Sensitive < 160 fg PGE, per well

Samples/Kit 39 or 231 in Duplicate

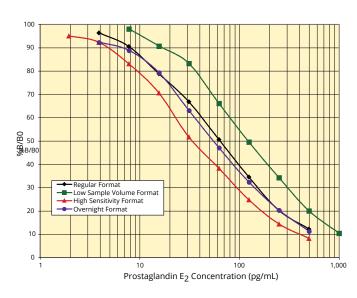
► Workflow Can be run in 2.5 hours or Overnight

► Versatile Measure PGE₂ in Mouse Serum without Extraction

Readout Colorimetric, 450 nm

#### SCIENTIFIC RELEVANCE

Eicosanoid signal transduction pathways are highly conserved and are involved in a number physiological processes. Prostaglandins are synthesized from arachidonic acid by cyclooxygenase (COX)-1 or -2, which convert the acid into PGH<sub>a</sub>. This is further processed by cytosolic or microsomal prostaglandin synthases to become PGE, or one of several other prostanoids. Prostacyclin is the major cyclooxygenase product in blood vessel walls and it is present in inflammatory fluids in similar concentrations to PGE, PGE, is produced by a wide variety of tissues and in several pathological conditions, including inflammation, arthritis, fever, tissue injury, endometriosis, and a variety of cancers. Other biological actions of PGE, include vasodilation, modulation of sleep/wake cycles, and facilitation of human



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immunodeficiency virus replication. It elevates cAMP levels, stimulates bone resorption, sodium excretion and renal hemodynamics, and has thermoregulatory effects.



## **Protein Kinase A (PKA) Colorimetric Activity Kit**

K027-H1 (1 Plate)

#### **FEATURES**

▶ Use Quantitate PKA Activity

Sample Cell Lysates, Purified Systems

Sensitive Most Sensitive Kit

Time to Answer 3 Hours

Species Species Independent

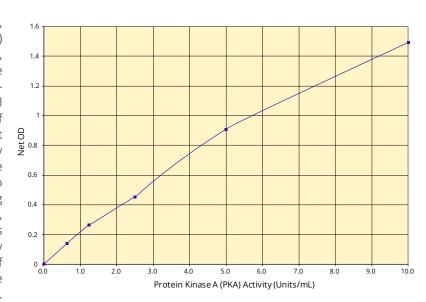
Samples/Kit 42 in Duplicate

Stability Fully Active PKA Standard, Storage at -20°C

► Readout Colorimetric, 450 nm

#### SCIENTIFIC RELEVANCE

The expressed PKA holoenzyme, comprising of two catalytic (C) and two regulatory (R) subunits, is activated when cAMP levels rise following stimulation of G Proteincoupled receptors and adenylyl cyclase. The phosphorylation of specific substrates by the C subunit of activated PKA is regulated by the subcellular localization of the enzyme through the binding to the scaffolding A kinase-anchoring proteins (AKAPs). In its inactive state, pseudosubstrate sequences on the R subunits stop the activity of the C subunits. Upon binding of cAMP to the R subunits, the active monomeric C subunits are released.



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PKA shares substrate specificity with Akt (PKB) and PKC. Substrates that are phosphorylated by PKA include Bad (Ser<sup>155</sup>), CREB (Ser<sup>133</sup>), and GSK-3 (GSK-3 GSK-3 GSK-3 GSK-3 GSK-3 Ser<sup>9</sup>). PKA is a pivotal kinase involved in cancer, vasodilation, metabolic processes, etc.



# **Enzyme Inhibitors and Activators**

### PHOSPHODIESTERASE INHIBITOR

| IBMX      | Pan-specific inhibitor of PDEs | 100 mg & 1G | P019-100MG/1G | N TEN   |
|-----------|--------------------------------|-------------|---------------|---|
| BRL-50481 | Specific inhibitor of PDE7     | 10 & 50 mg  | P020-10/50MG  | H <sub>3</sub> C Q Q N(CH <sub>3</sub> ) <sub>2</sub> |

### **STING INHIBITORS**

| H-151 | Irreversible STING inhibitor | 10 & 50 mg | P023-10/50MG |  |
|-------|------------------------------|------------|--------------|--|
| DMXAA | STING inhibitor              | 5 & 25 mg  | P024-5/25MG  |  |

## **CANCER CHEMOPROTECTANT**

| Xanthohumol | Inhibits the function of VCP | 10 & 50 mg | P021-10/50MG | он о<br>но осн <sub>з</sub> он |
|-------------|------------------------------|------------|--------------|--------------------------------|
|-------------|------------------------------|------------|--------------|--------------------------------|

### NAD BIOSYNTHESIS INHIBITOR

| FK-866, HCl | Specific inhibitor of NAMPT | 5 & 25 mg | P006-5/25MG | HO |
|-------------|-----------------------------|-----------|-------------|----|
|-------------|-----------------------------|-----------|-------------|----|



