Product Manual

cAMP ELISA Kit (Colorimetric)

Catalog Numbers

STA-500	96 assays
STA-500-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

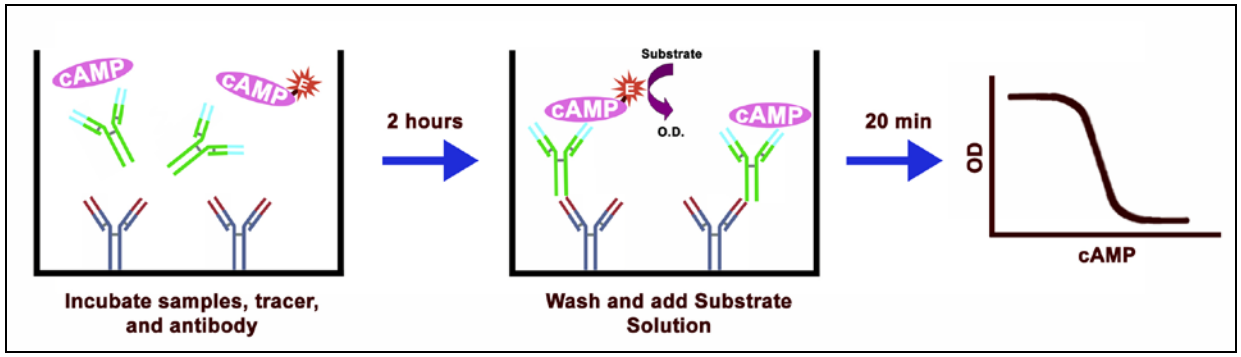
Adenosine 3',5'-cyclic monophosphate (cAMP) is a ubiquitous second messenger involved in various cellular activities in many cell and tissue types. It is converted from adenosine triphosphate (ATP) via adenylyl cyclases (AC), and is inactivated by hydrolysis to 5´-AMP by the actions of phosphodiesterases. cAMP may affect cellular function through several different mechanisms including the activation of cAMP-dependent Protein Kinase (PKA), Guanine Nucleotide Exchange Factors (GEFs), and Cyclic Nucleotide-gated (CNG) channels. PKA is a heterotetramer consisting of 2 regulatory (R) subunits and 2 catalytic (C) subunits. Two cAMP molecules bind cooperatively to 2 sites on each R subunit, releasing the active C subunit monomers to phosphorylate a range of downstream substrates. GEFs facilitate the exchange of GDP for GTP and, therefore, promote the activity of G proteins. Exchange Protein Activated by cAMP (Epac) 1 and 2 are GEFs activated upon binding to cAMP. Epac 1 and 2 have been implicated in regulating the activity of the small GTPase Rap-1 (26, 27). CNG channels are cation channels activated by cGMP and/or cAMP. These channels regulate membrane potential, and due to their Ca^{2+} permeability, can alter the levels of intracellular Ca^{2+}.

Cell Biolabs' cAMP ELISA Kit is a competitive enzyme immunoassay designed to measure cAMP in cell culture supernatants, plasma, serum, saliva, urine, and cell lysates. The kit selectively measures cAMP levels without any significant cross reactivities to other nucleotides or cyclic nucleotides. Samples containing low cAMP levels may be acetylated (reagents provided) for increased sensitivity. Under non-acetylated conditions, the kit has a detection range of 1 to 1000 pmol/mL cAMP; however, under acetylated conditions, the sensitivity is enhanced (approx 100X) to a detection range of 10-2500 fmol/mL.

Assay Principle

An anti-Rabbit IgG polyclonal coating antibody is adsorbed onto a microtiter plate. Cyclic AMP present in the sample or standard competes with Peroxidase cAMP Tracer for plate binding, in the presence of Rabbit Anti-cAMP Polyclonal Antibody.

Following incubation and wash steps, any Peroxidase cAMP Tracer bound to the plate is detected with addition of Substrate Solution. The colored product formed in inversely proportional to the amount of cAMP present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from cAMP Standard and sample concentration is then determined.



Related Products

1. STA-501: cAMP ELISA Kit (Chemiluminescent)
2. STA-505: cGMP ELISA Kit (Colorimetric)
3. STA-506: cGMP ELISA Kit (Chemiluminescent)

Kit Components

1. Goat Anti-Rabbit Antibody Coated Plate (Part No. 250001): One strip well 96-well plate.
2. cAMP Standard (Part No. 250002): One 100 µL vial provided at 10 mM.
3. Rabbit Anti-cAMP Polyclonal Antibody (Part No. 250003): One 15 µL vial.
4. Peroxidase cAMP Tracer Conjugate (Part No. 250004): One 30 µL vial.
5. Assay Diluent (Part No. 250005): One 25 mL bottle.
6. Lysis Buffer (Part No. 250006): One 50 mL bottle.
7. 10X Wash Buffer (Part No. 250007): One 50 mL bottle.
8. Triethylamine (Part No. 250008): One 2 mL amber bottle.
9. Acetic Anhydride (Part No. 250009): One 1 mL amber bottle.
10. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
11. Stop Solution (Part. No. 310808): One 12 mL bottle.

Materials Not Supplied

1. Orbital plate shaker
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
6. Glass or polypropylene tubes for acetylated samples and standards

Storage

Store kit components at 4ºC. For longer term use, store the Rabbit Anti-cAMP Polyclonal Antibody at -20ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Rabbit Anti-cAMP Polyclonal Antibody: Immediately before use dilute the Rabbit Anti-cAMP Antibody 1:500 with Assay Diluent. Do not store diluted solutions.

- Peroxidase cAMP Tracer Conjugate: Immediately before use dilute the Peroxidase cAMP Tracer Conjugate 1:100 with Assay Diluent. Do not store diluted solutions.
- Acetylation Reagent: Preparation of the Acetylation Reagent should be done in glass tubes and in a fume hood. The Acetylation Reagent is made by mixing Acetic Anhydride with Triethylamine at a 1:2 ratio (example: 0.5 mL Acetic Anhydride + 1 mL Triethylamine). Use the reagent within 60 minutes of preparation.

  Caution: The components of this reagent are known to be caustic, corrosive, flammable, and lachrymators. Use appropriate protection when handling.

Preparation of cAMP Standards (Non-Acetylated Version)

1. Thaw the cAMP Standard at room temperature and mix thoroughly by pipetting (cAMP can precipitate when frozen but will redissolve when mixed well). Freshly prepare a dilution series of cAMP Standard in the concentration range of 100 µM – 100 pM by diluting the cAMP Standard in Lysis Buffer (Table 1).

Standard Tubes	cAMP Standard (µL)	Lysis Buffer (µL)	cAMP Concentration
1	10	990	100 µM
2	20 of Tube #1	180	10 µM
3	20 of Tube #2	180	1 µM
4	20 of Tube #3	180	100 nM
5	20 of Tube #4	180	10 nM
6	20 of Tube #5	180	1 nM
7	20 of Tube #6	180	100 pM
8	0	180	0

Table 1. Preparation of Non-Acetylated cAMP Standard Curve

Preparation of Samples (Non-Acetylated Version)

- Urine, Serum, Plasma and Culture Medium Samples: Urine, serum and plasma may be tested directly with 1:200 to 1:1000 dilutions in Lysis Buffer. Culture medium can also be tested with 1:10 to 1:200 dilutions in Lysis Buffer.
  Note: RPMI medium may contain >350 fmol/µL cAMP).
- Cell Samples: Aspirate medium. Add 1 ml of Lysis Buffer for every 35 cm^2 of surface area. Incubate at 4ºC for 20 minutes. Scrape cells off the surface with a cell scraper. Dissociate sample by pipetting up and down until suspension is homogeneous. Transfer to a centrifuge tube and centrifuge at top speed for 10 min. The supernatant can be assayed directly. Protein concentration >1 mg/ml is recommended for reproducible results.
- Tissue Samples: Cyclic nucleotides may be metabolized quickly in tissue, so it is important to rapidly freeze tissues after collection (e.g., using liquid nitrogen). Weigh the frozen tissue and add 5-10 µL of Lysis Buffer per mg of tissue. Homogenize the sample on ice using a Polytron-type

homogenizer. Spin at top speed for 5 min and collect the supernatant. The supernatant may be assayed directly.

Preparation of cAMP Standards (Acetylated Version)

Note: Samples containing low cAMP levels may be acetylated for increased sensitivity (approx 100-fold), although overall assay values will be lowered 2-3 fold.

1. Thaw the cAMP Standard at room temperature and mix thoroughly by pipetting (cAMP can precipitate when frozen but will redissolve when mixed well). In glass or polypropylene tubes, freshly prepare a dilution series of cAMP Standard in the concentration range of 10 nM – 2.4 pM by diluting the cAMP Standard in Lysis Buffer (Table 2).

   Note: The kit cAMP Standard, provided at 10 mM, must first be aggressively diluted to achieve the desired range. A series of 1:100 dilutions are suggested (denoted Stock A and B). Stock A and B are <u>not</u> to be included in the standard curve; only tubes 1-8 should be transferred.

Standard Tubes	cAMP Standard (µL)	Lysis Buffer (µL)	Final cAMP Concentration
Stock A	10 of cAMP Standard (10 mM)	990	100 uM
Stock B	10 of Stock A	990	1 uM
1	10 of Stock B	990	10 nM
2	100 of Tube #1	300	2.5 nM
3	100 of Tube #2	300	625 pM
4	100 of Tube #3	300	156 pM
5	100 of Tube #4	300	39 pM
6	100 of Tube #5	300	9.8 pM
7	100 of Tube #6	300	2.4 pM
8	0	300	0

Table 2. Preparation of Acetylated cAMP Standard Curve

2. In the hood, transfer 200 µL of tubes 1-8 to new tubes and acetylate each by adding 10 µL of Acetylation Reagent (see Preparation of Reagents). Mix well and use within 30 minutes.

Preparation of Samples (Acetylated Version)

Note: Samples containing low cAMP levels may be acetylated for increased sensitivity (approx. 100-fold), although overall assay values will be lowered 2-3 fold.

- Urine, Serum, Plasma and Culture Medium Samples: Urine, serum and plasma may be tested directly with 1:200 to 1:1000 dilutions in Lysis Buffer. Culture medium can also be tested with 1:10 to 1:200 dilutions in Lysis Buffer. To acetylate the sample, add 10 µL of Acetylation Reagent (see Preparation of Reagents) to 200 µL of sample in a glass or polypropylene tube. Mix well and use within 30 minutes.
  Note: RPMI medium may contain >350 fmol/µL cAMP).
- Cell Samples: Aspirate medium. Add 1 ml of Lysis Buffer for every 35 cm^2 of surface area. Incubate at 4ºC for 20 minutes. Scrape cells off the surface with a cell scraper. Dissociate sample

by pipetting up and down until suspension is homogeneous. Transfer to a centrifuge tube and centrifuge at top speed for 10 min. The supernatant can be assayed directly. Protein concentration >1 mg/ml is recommended for reproducible results. To acetylate the sample, add 10 µL of Acetylation Reagent (see Preparation of Reagents) to 200 µL of sample in a glass or polypropylene tube. Mix well and use within 30 minutes.

- Tissue Samples: Cyclic nucleotides may be metabolized quickly in tissue, so it is important to rapidly freeze tissues after collection (e.g., using liquid nitrogen). Weigh the frozen tissue and add 5-10 µL of Lysis Buffer per mg of tissue. Homogenize the sample on ice using a Polytron-type homogenizer. Spin at top speed for 5 min and collect the supernatant. The supernatant may be assayed directly. To acetylate the sample, add 10 µL of Acetylation Reagent (see Preparation of Reagents) to 200 µL of sample in a glass or polypropylene tube. Mix well and use within 30 minutes.

Assay Protocol

Important Note: Add reagents to the plate gently using a multichannel pipette. To avoid the creation of bubbles in the well, do not mix by pipetting.

1. Prepare and mix all reagents thoroughly before use.
2. Each cAMP sample, cAMP Standard, and blank should be assayed in duplicate.

   Note: cAMP samples must be compared with corresponding standards (i.e. acetylated samples compared with acetylated standards; non-acetylated samples with non-acetylated standards).
3. Add 50 µL of cAMP sample or standard (acetylated or non-acetylated) to the Goat Anti-Rabbit Antibody Coated Plate.
4. Add 25 µL of diluted Peroxidase cAMP Tracer Conjugate (see Preparation of Reagents Section) to each tested well.
5. Add 50 µL of diluted Rabbit Anti-cAMP Polyclonal Antibody (see Preparation of Reagents Section) to each tested well.
6. Cover with a Plate Cover and incubate at room temperature for 2 hours with shaking.
7. Remove Plate Cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature for 5-20 minutes on an orbital shaker.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical cAMP ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

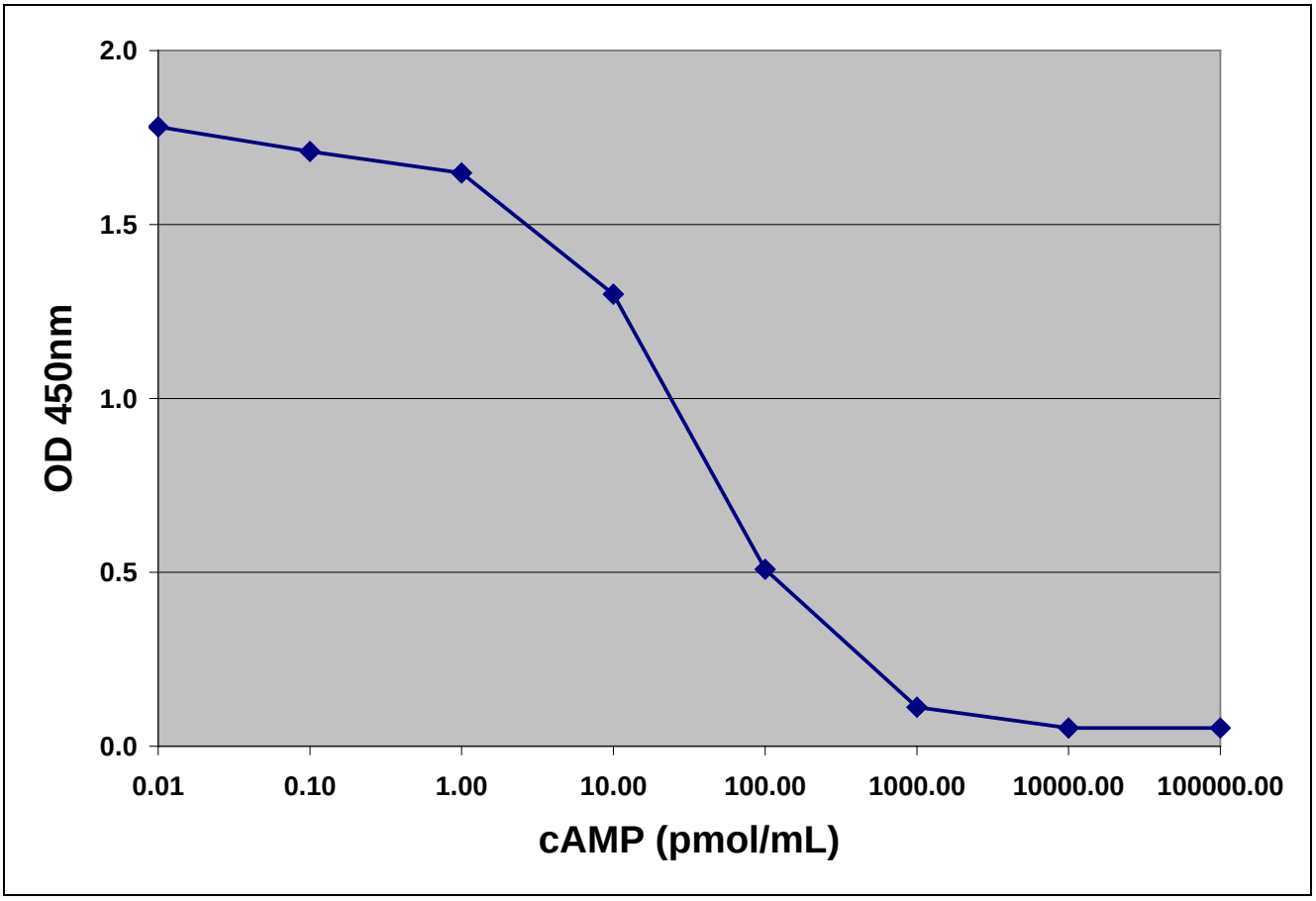


Figure 1: cAMP ELISA Standard Curve (Non-Acetylated Version)

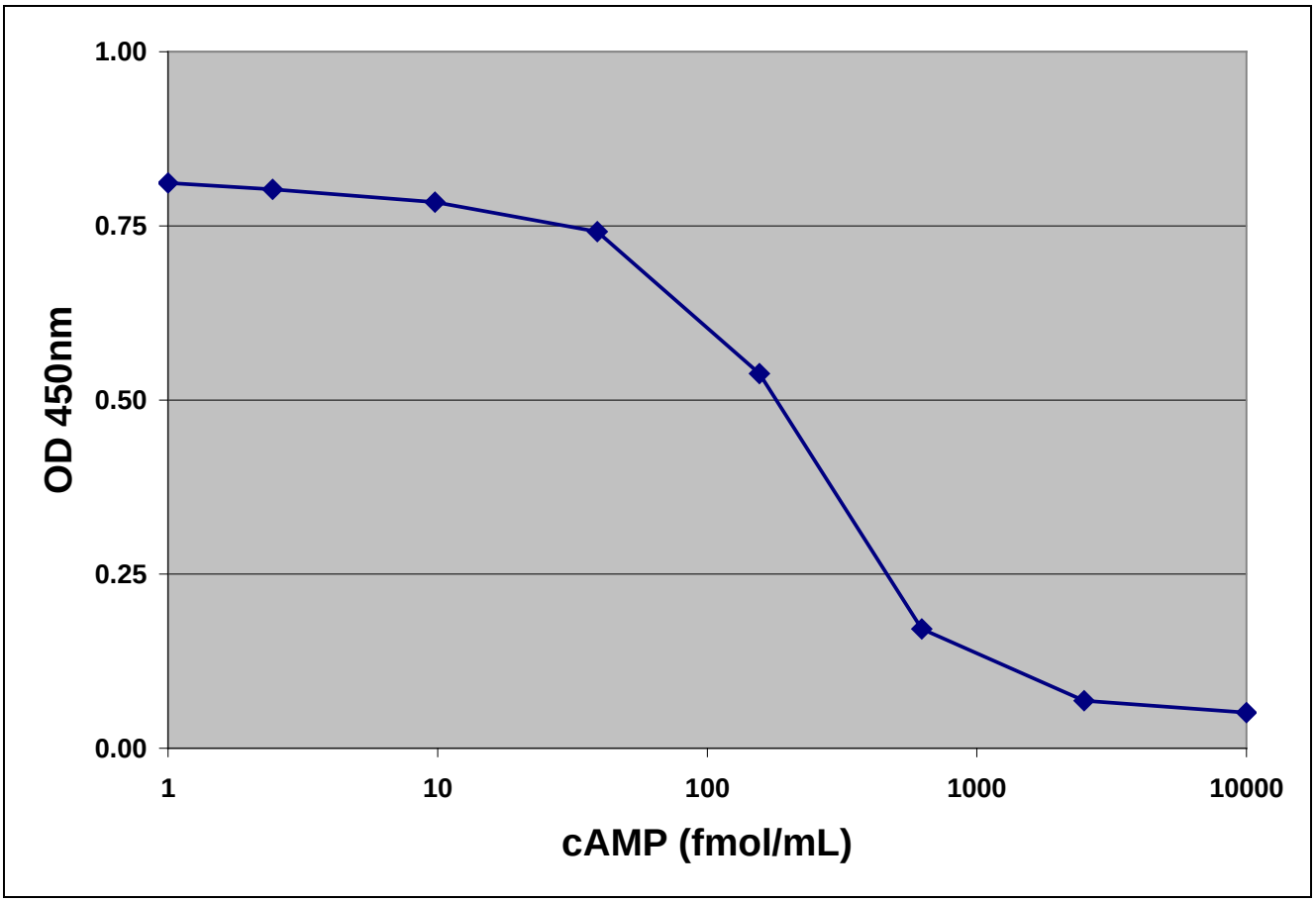


Figure 2: cAMP ELISA Standard Curve (Acetylated Version)

Cross reactivity of cAMP ELISA Kit

Compounds	Cross Reactivity
cAMP	100%
cGMP	<0.1%
AMP	<0.01%
ADP	<0.01%
ATP	<0.01%
GMP	<0.01%
GTP	<0.01%
CTP	<0.01%

References

1. Hanoune, J. et al. (1997) Mol. Cell. Endocrinol. 128:179.
2. Patel, T.B. et al. (2001) Gene 269:13.
3. Smit, M.J. and R. Iyengar (1998) Adv. Second Messenger Phosphoprotein Res. 32:1.
4. Sunahara, R.K. et al. (1996) Annu. Rev. Pharmacol. Toxicol. 36:461.
5. Sunahara, R.K. and R. Taussig (2002) Mol Interv 2:168.
6. Taylor, S.S. (1989) J. Biol. Chem. 264:8443.
7. Seino, S. and T. Shibasaki (2005) Physiol. Rev. 85:1303.
8. de Rooij, J. et al. (2000) J. Biol. Chem. 275:20829.
9. de Rooij, J. et al. (1998) Nature 396:474.
10. Kaupp, U.B. and R. Seifert (2002) Physiol. Rev. 82:769.

Recent Product Citations

1. Meng, W. et al. (2017). Rheb Inhibits Beigeing of White Adipose Tissue via PDE4D5-dependent Down-regulation of the cAMP-PKA Signaling Pathway. *Diabetes*. pii: db160886. doi: 10.2337/db16-0886.
2. Iyer, S.V. et al. (2016). Genome-wide RNAi screening identifies TMIGD3 isoform1 as a suppressor of NF-kB and osteosarcoma progression. *Nat. Commun*.**7**:13561.
3. Zhao, W. et al. (2016). In silico modelling of novel drug ligands associated with abnormal tau phosphorylation: Implications for concussion associated tauopathy intervention. *J Cell Biochem*. doi:10.1002/jcb.25521.
4. He, T. et al. (2015). Role of prostacyclin signaling in endothelial production of soluble amyloid precursor protein-α in cerebral microvessels. *J Cereb Blood Flow Metab.* doi:10.1177/0271678X15618977.
5. Omosun, Y. et al. (2015). IL-10 modulates antigen presentation by dendritic cells through regulation of NLRP3 inflammasome assembly during Chlamydia infection. *Infect Immun.* doi:10.1128/IAI.00993-15.
6. Rose, S. J. et al. (2015). A new knock-in mouse model of l-DOPA-responsive dystonia. *Brain*. doi:10.1093/brain/awv212.
7. Liu, X. et al. (2015). β-Arrestin-biased signaling mediates memory reconsolidation. *Proc Natl Acad Sci U S A*. doi: 10.1073/pnas.1421758112.
8. Cortés, V. et al. (2015). Metabolic effects of cholecystectomy: gallbladder ablation increases basal metabolic rate through g-protein coupled bile acid receptor gpbar1-dependent mechanisms in mice. *PLoS One*. **10**: e0118478.
9. Terunuma, M. et al. (2015). Purinergic receptor activation facilitates astrocytic GABA B Receptor calcium signalling. *Neuropharmacology*. **88**:74-81.
10. McRobie, H. R. et al. (2014). Agouti signalling protein is an inverse agonist to the wildtype and agonist to the melanic variant of the melanocortin-1 receptor in the grey squirrel (Sciurus carolinensis). *FEBS Lett.* **588**:2335-2343.
11. Trehan, A. et al. (2014). CANDLES, an assay for monitoring GPCR induced cAMP generation in cell cultures. *Cell Commun Signal.* **12**:70.

12. Smith, E. P. et al. (2014). The role of β cell glucagon-like peptide-1 signaling in glucose regulation and response to diabetes drugs. *Cell metab.* **19**:1050-1057.
13. Tian, L. et al. (2014). TSH stimulates the proliferation of vascular smooth muscle cells. *Endocrine*. **46**:651-658.
14. Yong, Y. et al. (2014). Electromagnetic fields promote osteogenesis of rat mesenchymal stem cells through the PKA and ERK1/2 pathways. *J Tissue Eng Regen Med*. doi: 10.1002/term.1864.
15. Jones, A. et al. (2014). Human macrophage SCN5A activates an innate immune signaling pathway for antiviral host defense. *J Biol Chem*. **289**:35326-35340.
16. Chen, X. et al. (2014). Identification of serine 348 on the apelin receptor as a novel regulatory phosphorylation site in apelin-13-induced G Protein-independent biased signaling. *J Biol Chem*. **289**:31173-31187.
17. Peterson, J. R. et al. (2014). Treatment of heterotopic ossification through remote ATP hydrolysis. *Sci Transl Med*. **6**:255ra132.
18. Rachmin, I. et al. (2014). Erbin is a negative modulator of cardiac hypertrophy. *PNAS* **111**:5902-5907.
19. Meyer, R et al. (2013). GPR37 and GPR37L1 are receptors for the neuroprotective and glioprotective factors prosaptide and prosaposin. *PNAS*. 10.1073-PNAS.1219004110.
20. Sun, Z. et al. (2011). The WD40 repeat protein WDR26 binds Gßγ and promotes Gßγ-dependent signal transduction and leukocyte migration. *J. Biol. Chem.* **286**:-43902-43912.
21. McCoy, K.L. el al. (2010). PAR1 and PAR2 couple to overlapping and distinct sets of G Proteins and linked signaling pathways to differentially regulate cell physiology. *Mol. Pharmacol.* **77**:1005-1015.
22. Chen, M. et al. (2010). Involvement of cAMP in nerve growth factor-triggered p35/Cdk5 activation and differentiation in PC12 cells. *Am J. Physiol Cell Physiol.* **299**:C516-C527.

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