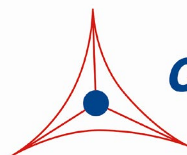
Product Manual

# 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



CELL BIOLABS, INC.
*Creating Solutions for Life Science Research*

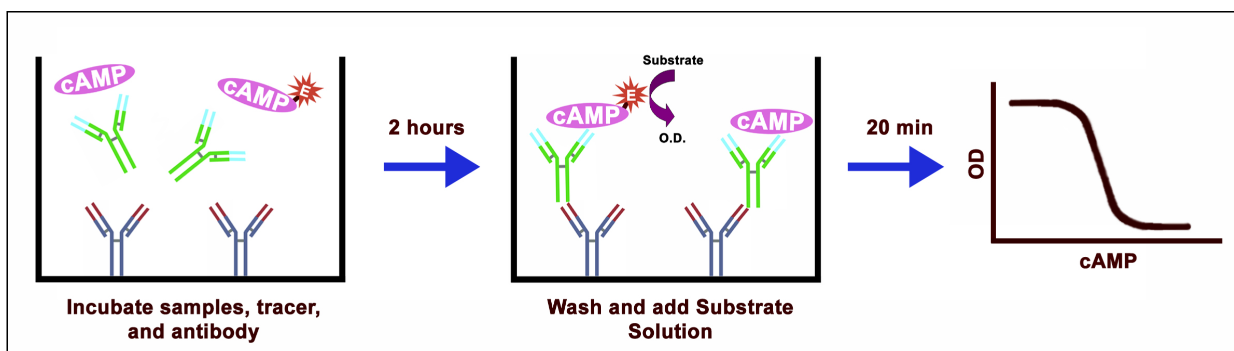
## Introduction

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 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.






CELL BIOLABS, INC.
Creating Solutions for Life Science Research

## Related Products

1. STA-415: ROCK Activity Immunoblot Kit
2. STA-416: 96-Well ROCK Activity Assay Kit
3. STA-501: cAMP ELISA Kit (Chemiluminescent)

## Kit Components (shipped at room temperature)

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 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. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
3. Glass or polypropylene tubes for acetylated samples and standards

## Storage

Store both the Rabbit Anti-cAMP Antibody and the Peroxidase cAMP Tracer Conjugate at -20ºC.
Store all other kit components at 4ºC.

## Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Rabbit Anti-cAMP 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 (nM) |
|---|---|---|---|
| 1 | 10 | 990 | 100,000 |
| 2 | 20 of Tube #1 | 180 | 10,000 |
| 3 | 20 of Tube #2 | 180 | 1000 |
| 4 | 20 of Tube #3 | 180 | 100 |
| 5 | 20 of Tube #4 | 180 | 10 |
| 6 | 20 of Tube #5 | 180 | 1 |
| 7 | 20 of Tube #6 | 180 | 0.1 |
| 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 not 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 (nM) |
|---|---|---|---|
| Stock A | 10 of cAMP Standard (10 mM) | 990 | 100,000 |
| Stock B | 10 of Stock A | 990 | 1000 |
| 1 | 10 of Stock B | 990 | 10 |
| 2 | 100 of Tube #1 | 300 | 2.5 |
| 3 | 100 of Tube #2 | 300 | 0.625 |
| 4 | 100 of Tube #3 | 300 | 0.156 |
| 5 | 100 of Tube #4 | 300 | 0.039 |
| 6 | 100 of Tube #5 | 300 | 0.010 |
| 7 | 100 of Tube #6 | 300 | 0.0025 |
| 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 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.

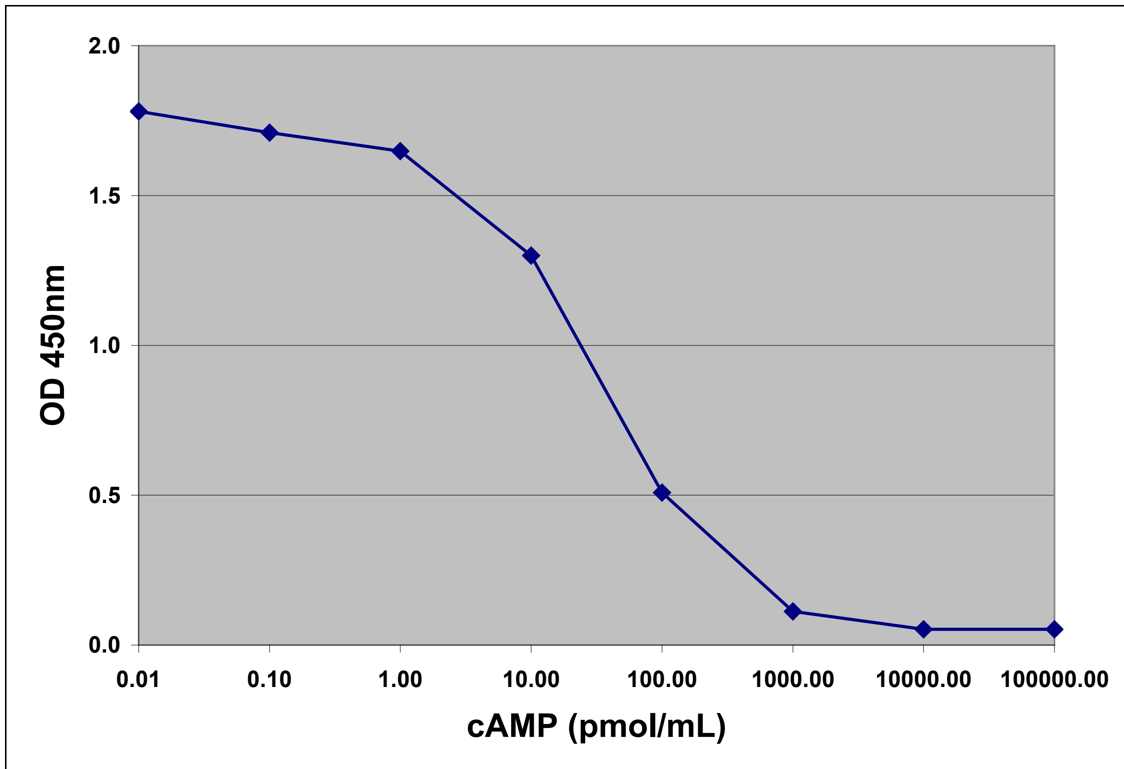



**Figure 1: cAMP ELISA Standard Curve (Non-Acetylated Version)**

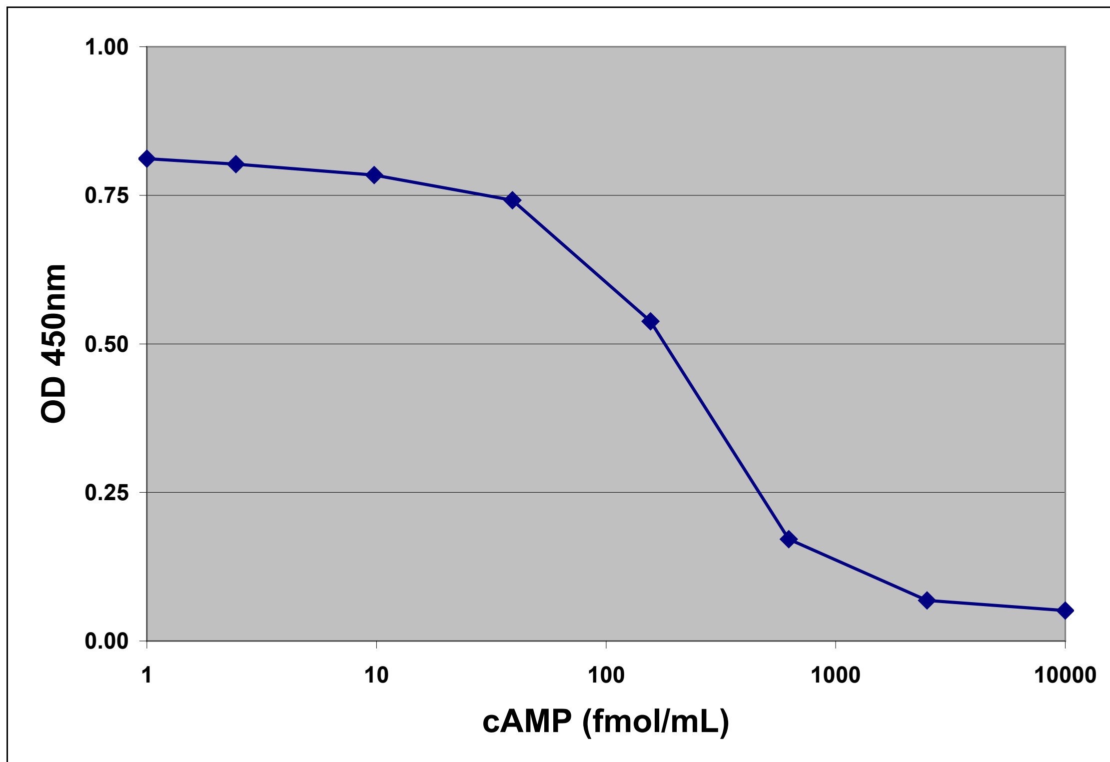



**Figure 2: cAMP ELISA Standard Curve (Acetylated Version)**



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

**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. Mystek, P. et al. (2025). The polybasic puzzle: how N-terminal charges modulate Gαi1 membrane behavior and signaling output. *Cell Commun Signal*. doi: 10.1186/s12964-025-02541-0.
2. Manoel, D. et al. (2025). Functional Characterisation and cAMP-Mediated Rescue of a Novel Truncating AVPR2 Mutation Causing Nephrogenic Diabetes Insipidus. *Am J Physiol Endocrinol Metab*. doi: 10.1152/ajpendo.00325.2025.
3. Kavinda, M.H.D. et al. (2024). Anti–melanogenic properties of FBCC–EP850 derived from Carex pumila Thunb. *Asian Pac J Trop Biomed*. **14**(11):477-485. doi: 10.4103/apjtb.apjtb_353_24.
4. Rysiewicz, B. et al. (2023). Beyond the G protein α subunit: investigating the functional impact of other components of the Gαi3 heterotrimers. *Cell Commun Signal*. **21**(1):279. doi: 10.1186/s12964-023-01307-w.
5. Jin, Z. et al. (2023). Vertical sleeve gastrectomy-derived gut metabolite licoricidin activates beige fat thermogenesis to combat obesity. *Theranostics*. **13**(9):3103-3116. doi: 10.7150/thno.81893.
6. Kotlarczyk, A.M. et al. (2023). How Is Arachidonic Acid Metabolism in the Uterus Connected with the Immune Status of Red Deer Females (Cervus elaphus L.) in Different Reproductive Stages? *Int J Mol Sci*. **24**(5):4771. doi: 10.3390/ijms24054771.
7. Athapaththu, A.M.G.K. et al. (2023). Pinostrobin Suppresses the α-Melanocyte-Stimulating Hormone-Induced Melanogenic Signaling Pathway. *Int J Mol Sci*. **24**(1):821. doi: 10.3390/ijms24010821.
8. Al-Ghafari, A. et al. (2022). Cyclic AMP and calcium signaling are involved in antipsychotic-induced diabetogenic effects in isolated pancreatic β cells of CD1 mice. *Int J Health Sci (Qassim)*. **16**(5):9-20.

9. Lee, D. & PyoIn, Y. (2021). Vitro Anti-Obesity Effects of Raw Garlic and Pickled Garlic. *J Korean Med Obes Res*. **21**:69-79. doi: 10.15429/jkomor.2021.21.2.69.
10. Lee, S.J. et al. (2021). Anti-Obesity Effect of α-Cubebenol Isolated from Schisandra chinensis in 3T3-L1 Adipocytes. *Biomolecules*. **11**(11):1650. doi: 10.3390/biom11111650.
11. Boby, N. et al. (2021). Protective Effect of Pyrus ussuriensis Maxim. Extract against Ethanol-Induced Gastritis in Rats. *Antioxidants*. **10**(3):439. doi: 10.3390/antiox10030439.
12. Huang, J. et al. (2020). The odorant receptor OR2W3 on airway smooth muscle evokes bronchodilation via a cooperative chemosensory tradeoff between TMEM16A and CFTR. *Proc Natl Acad Sci U S A*. doi: 10.1073/pnas.2003111117.
13. Ridzwan, N. et al. (2020). Pomegranate-derived anthocyanin regulates MORs-cAMP/CREB-BDNF pathways in opioid-dependent models and improves cognitive impairments. *J Ayurveda Integr Med*. S0975-9476(18)30683-1. doi: 10.1016/j.jaim.2019.12.001.
14. Zhong, Y. et al. (2020). Berberine Attenuates Hyperglycemia by Inhibiting the Hepatic Glucagon Pathway in Diabetic Mice. *Oxid Med Cell Longev*. **2020**:6210526. doi: 10.1155/2020/6210526.
15. Molagoda, I.M.N. et al. (2020). Ethanolic Extract of Hippocampus abdominalis Exerts Anti-Melanogenic Effects in B16F10 Melanoma Cells and Zebrafish Larvae by Activating the ERK Signaling Pathway. *Cosmetics*. **7**(1):1-14. doi: 10.3390/cosmetics7010001.
16. Wójcik-Pszczoła, K. et al. (2019). Novel phosphodiesterases inhibitors from the group of purine-2,6-dione derivatives as potent modulators of airway smooth muscle cell remodelling. *Eur J Pharmacol.* doi: 10.1016/j.ejphar.2019.172779.
17. Mystek, P. et al. (2019). Gγ and Gα Identity Dictate a G-Protein Heterotrimer Plasma Membrane Targeting. *Cells*. **8**(10). pii: E1246. doi: 10.3390/cells8101246.
18. Meng, W. et al. (2019). Rheb promotes brown fat thermogenesis by Notch-dependent activation of the PKA signaling pathway. *J Mol Cell Biol*. pii: mjz056. doi: 10.1093/jmcb/mjz056.
19. Chen, C. et al. (2019). 5'-Iodotubercidin represses insulinoma-associated-1 expression, decreases cAMP levels, and suppresses human neuroblastoma cell growth. *J Biol Chem*. **294**(14):5456-5465. doi: 10.1074/jbc.RA118.006761.
20. d'Uscio, L.V. et al. (2019). Vascular phenotype of amyloid precursor protein-deficient mice. *Am J Physiol Heart Circ Physiol*. doi: 10.1152/ajpheart.00539.2018.
21. Gogola, J. et al. (2019). Persistent endocrine-disrupting chemicals found in human follicular fluid stimulate the proliferation of granulosa tumor spheroids via GPR30 and IGF1R but not via the classic estrogen receptors. *Chemosphere*. **217**:100-110. doi: 10.1016/j.chemosphere.2018.11.018.
22. Mamat, N. et al. (2018). Potential anti-vitiligo properties of cynarine extracted from Vernonia anthelmintica (L.) Willd. *Int J Mol Med*. **42**(5):2665-2675. doi: 10.3892/ijmm.2018.3861.
23. Wang, W. et al. (2018). Decreased cAMP Level and Decreased Downregulation of β1-Adrenoceptor Expression in Therapeutic Hypothermia-Resuscitated Myocardium Are Associated With Improved Post-Resuscitation Myocardial Function. *J Am Heart Assoc*. **7**(6). pii: e006573. doi: 10.1161/JAHA.117.006573.
24. He, T. et al. (2017). Impairment of amyloid precursor protein alpha-processing in cerebral microvessels of type 1 diabetic mice. *J Cereb Blood Flow Metab*. 271678X17746981. doi: 10.1177/0271678X17746981.
25. Kang, J.A. et al. (2018). Gamma-irradiated black ginseng extract inhibits mast cell degranulation and suppresses atopic dermatitis-like skin lesions in mice. *Food Chem Toxicol*. **111**:133-143. doi: 10.1016/j.fct.2017.11.006.

26. 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.
27. 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.
28. 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.
29. 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.
30. 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.

## Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

## Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858 271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2007-2026: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.