Datasheet for ABIN2345105

# Cathelicidin ELISA Kit

23 Publications

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## Overview

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| Quantity: | 96 tests |
| Target: | Cathelicidin (CAMP) |
| Reactivity: | Chemical |
| Method Type: | Competition ELISA |
| Application: | ELISA |

## Product Details

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| Sample Type: | Plasma, Urine |
| Analytical Method: | Quantitative |
| Detection Method: | Colorimetric |
| Sensitivity: | 1 pmol/mL |
| Characteristics: | 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. |
| Components: | 1. Goat Anti-Rabbit Antibody Coated Plate : One strip well 96-well plate.<br>2. cAMP Standard : One 100 µL vial provided at 10 mM.<br>3. Rabbit Anti-cAMP Polyclonal Antibody : One 15 µL vial.<br>4. Peroxidase cAMP Tracer Conjugate : One 30 µL vial. |

## Product Details

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| | 5. Assay Diluent : One 25 mL bottle.<br>6. Lysis Buffer : One 50 mL bottle.<br>7. 10X Wash Buffer : One 50 mL bottle.<br>8. Triethylamine : One 2 mL amber bottle.<br>9. Acetic Anhydride : One 1 mL amber bottle.<br>10. Substrate Solution : One 12 mL amber bottle.<br>11. Stop Solution (Part. No. 310808): One 12 mL bottle. |
| Material not included: | 1. Orbital plate shaker<br>2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips<br>3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips<br>4. Multichannel micropipette reservoir<br>5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)<br>6. Glass or polypropylene tubes for acetylated samples and standards |

## Target Details

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| Target: | Cathelicidin (CAMP) |
| Alternative Name: | cAMP (CAMP Products) |
| Target Type: | Chemical |
| Background: | 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 Ca2+ permeability, can alter the levels of intracellular Ca2+. |
| Pathways: | Cellular Response to Molecule of Bacterial Origin |

## Application Details

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| Application Notes: | Optimal working dilution should be determined by the investigator. |
| Comment: | • Sensitivity as low as 1 pmol/mL<br>• Suitable for use with cell and tissue lysates, urine, plasma, or culture medium<br>• Convenient strip-well plate format |
| Plate: | Uncoated |
| Protocol: | 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. |
| Reagent Preparation: | • 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.<br>• Rabbit Anti-cAMP Polyclonal Antibody: Immediately before use dilute the Rabbit Anti-cAMP Antibody 1:500 with Assay Diluent. Do not store diluted solutions. 3<br>• Peroxidase cAMP Tracer Conjugate: Immediately before use dilute the Peroxidase cAMP Tracer Conjugate 1:100 with Assay Diluent. Do not store diluted solutions.<br>• 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). Lysis Buffer cAMP Standard Tubes cAMP Standard (µL) (µL) 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. |
| Assay Procedure: | 1. Prepare and mix all reagents thoroughly before use.<br>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).<br>3. Add 50 µL of cAMP sample or standard (acetylated or non-acetylated) to the Goat Anti-Rabbit |

## Application Details

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. 6

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| Restrictions: | For Research Use only |

## Handling

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| Storage: | 4 °C/-20 °C |
| Storage Comment: | Store kit components at 4°C. For longer term use, store the Rabbit Anti-cAMP Polyclonal Antibody at -20°C. |

## Publications

Product cited in:

Meng, Liang, Chen, Luo, Bai, Li, Zhang, Xiao, He, Zhang, Xu, Xiao, Liu, Hu, Liu: "Rheb Inhibits Beigeing of White Adipose Tissue via PDE4D5-dependent Down-regulation of the cAMP-PKA Signaling Pathway." in: **Diabetes**, (2017) (PubMed).

Zhao, Ho, Wang, Bi, Yemul, Ward, Freire, Mazzola, Brathwaite, Mezei, Sanchez, Elder, Pasinetti: " In Silico Modelling of Novel Drug Ligands Associated with Abnormal Tau Phosphorylation: Implications for Concussion Associated Tauopathy Intervention." in: **Journal of cellular biochemistry**, (2016) (PubMed).

Iyer, Ranjan, Elias, Parrales, Sasaki, Roy, Umar, Tawfik, Iwakuma: "Genome-wide RNAi screening identifies TMIGD3 isoform1 as a suppressor of NF-κB and osteosarcoma progression." in: **Nature communications**, Vol. 7, pp. 13561, (2016) (PubMed).

Trehan, Rotgers, Coffey, Huhtaniemi, Rivero-Müller: "CANDLES, an assay for monitoring GPCR induced cAMP generation in cell cultures." in: **Cell communication and signaling : CCS**, Vol. 12, pp. 70, (2015) (PubMed).

Cortés, Amigo, Zanlungo, Galgani, Robledo, Arrese, Bozinovic, Nervi: "Metabolic effects of cholecystectomy: gallbladder ablation increases basal metabolic rate through G-protein coupled bile acid receptor Gpbar1-dependent mechanisms in mice." in: **PLoS ONE**, Vol. 10, Issue 3, pp. e0118478, (2015) (PubMed).

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