VALIDATION DATA

Cyclic UMP ELISA Kit

Item No. 502490

The Cyclic UMP ELISA Kit was assessed using a variety of tests and procedures to validate its performance for the quantitative measurement of cyclic uridine 3',5'-monophosphate (cUMP) in biological matrices commonly used with this kit. The validation experiments were designed to evaluate assay sensitivity, working range, precision, accuracy, and matrix compatibility under the conditions described in the kit booklet.

The data presented in this document describe the performance characteristics of the assay and are intended to assist users in experimental planning and interpretation of results.

Parameter	Performance Metric
Detection method	Colorimetric (405-420 nm)
Assay duration	3 hours
Lower Limit of Detection (LLOD)	0.11 nM
Sample volume	50 µl
Species compatibility	Bacteria
Validated samples	Bacterial lysates from *E. coli* and *P. aeruginosa*

Assay Range and Sensitivity

The assay range is 0.14-300 nM when determined under the conditions described in the kit protocol.

The lower limit of detection (LLOD) represents the lowest concentration that can be reliably distinguished from the background signal. The LLOD for the assay is 0.11 nM cUMP.

The sensitivity of this assay is determined as a value corresponding to 80% B/B_0 of the standard curve. The sensitivity of the assay is 0.95 nM cUMP.

WARNING
THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA
This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

WARRANTY AND LIMITATION OF REMEDY
Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

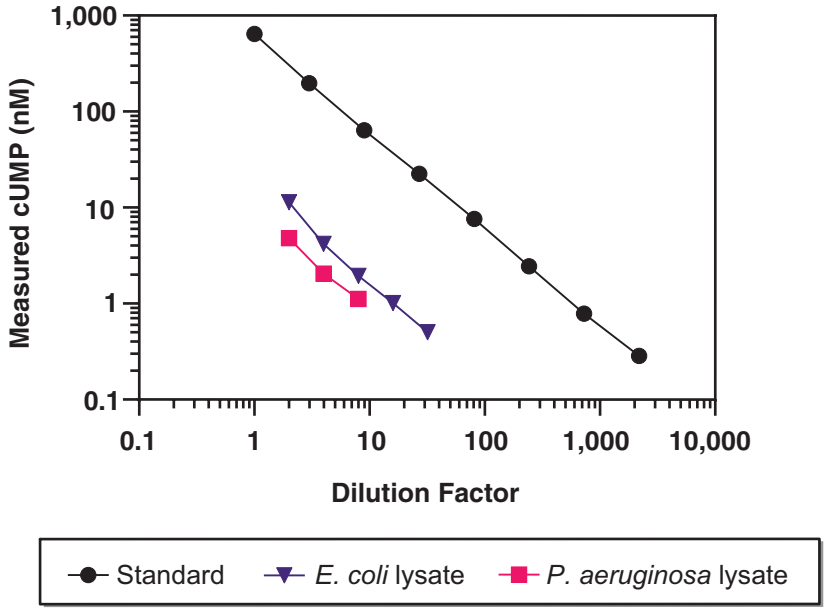
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Parallelism

Parallelism experiments are performed to evaluate the relationship between the dose-response curve of various samples and the calibration standard series to show that the native analyte in the sample matrix performs similarly to the reference standard. Parallelism experiments also assess the effect of sample matrices on analyte measurement and confirm that dilution of biological sample yields proportional changes in measured concentration.



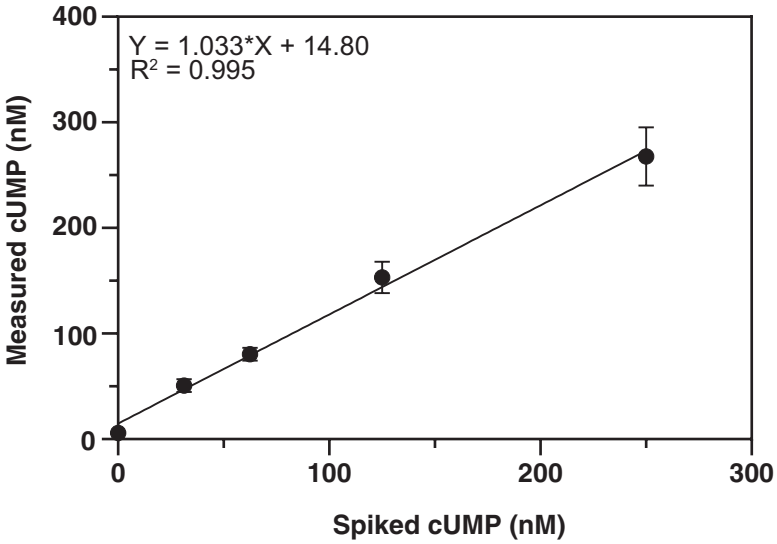
Parallelism of bacterial lysates. *P. aeruginosa* and *E. coli* lysates were diluted with Assay Buffer and analyzed using the Cyclic UMP ELISA Kit. Measured concentrations were plotted as a function of sample dilution.

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Spike and Recovery

Spike and recovery experiments are conducted to assess assay accuracy in the presence of complex biological matrices. In these experiments, known quantities of analyte are spiked into the sample. The samples are then processed according to the kit booklet, diluted with Assay Buffer, and analyzed using the assay. Measured concentrations are plotted as a function of spiked analyte concentration. The slope of the line multiplied by 100 indicates an average percent recovery. A recovered concentration between 80-120% of the expected concentration is acceptable. Values beyond this range could indicate the presence of assay interferants.



Spike and recovery of cUMP in *E. coli* lysates. *E. coli* lysate was spiked with cUMP, serially diluted with Assay Buffer, and evaluated using the Cyclic UMP ELISA Kit. The error bars indicate standard deviations from multiple dilutions of the same sample.

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Linearity

Linearity experiments are performed with biological samples spiked with high amounts of analyte to confirm that multiple dilutions of the sample yield similar measured concentrations of the analyte after dilution adjustment.

Dilution Factor	Measured cUMP, Dilution Adjusted (nM)	Linearity%
P. aeruginosa lysate spiked with 500 nM cUMP		
12	540	100
24	620	115
48	630	117
96	655	121
E. coli lysate spiked with 500 nM cUMP		
15	499	100
30	491	98.3
60	478	95.7
120	485	97.2

NOTE: Linearity has been calculated using the following formula: % Linearity = (Observed concentration value, dilution adjusted/First observed concentration value in the dilution series, dilution adjusted) × 100

Linearity in bacterial lysates. *P. aeruginosa* and *E. coli* lysates were spiked with cUMP, serially diluted with Assay Buffer, and analyzed using the Cyclic UMP ELISA Kit.

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Precision

The coefficient of variation (CV) is a measurement of consistency between sample replicates. Precision was evaluated both within a single assay run (intra-assay) and across multiple runs (inter-assay) using controls prepared from *P. aeruginosa* and *E. coli* lysates.

Control	Measured cUMP (nM)	%CV
Control 1 (spiked *E. coli* lysate)	521	4.8
Control 2 (spiked *P. aeruginosa* lysate)	155	4.7
Control 3 (*E. coli* lysate)	6.8	4.3

Intra-Assay Precision: To assess intra-assay precision, a series of 24 replicate measurements were performed on the same day. The measured values and CVs are listed in the table above.

Control	Measured cUMP (nM)	%CV
Control 1 (spiked *E. coli* lysate)	579	10.3
Control 2 (spiked *P. aeruginosa* lysate)	140	13.2
Control 3 (*E. coli* lysate)	11.6	13.7

Inter-Assay Precision: To assess inter-assay precision, a series of eight replicate measurements were performed on different days under the same experimental conditions. The measured values and CVs are listed in the table above.

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