Automation of a GPCR Cell-Based Functional Assay on the Cellular Workstation System using the LANCE cAMP Technology

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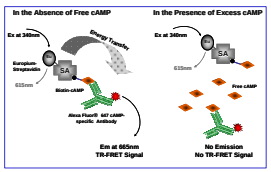
1 Introduction

- The Cellular Workstation is a fully integrated and automated workstation for performing cell-based assays. The system is comprised of the Evolution™ P3 Precision Pipetting Platform for reagent dispensing, the EnVision™ multilabel plate reader for detection, CataLyst Express robotic arm, and POLARA™ scheduling software. In addition to these core components, options such as Cytomat® microplate incubator, plate washer, and filtration unit can also be integrated. This workstation is a walk-away, easy-to use solution for cellular applications in the areas of target identification and validation, assay development, secondary screening and early ADME/Tox profiling.
- The LANCE cAMP assay is a homogenous time-resolved fluorescence resonance energy transfer (TR-FRET) immunoassay designed to measure cAMP produced upon modulation of adenylyl cyclase activity by GPCRs. We have automated the LANCE cAMP assay on the Cellular Workstation using a cell line expressing the 5-HT1a receptor, which is coupled to adenylyl cyclase through a Gαi-containing G protein that leads to the inhibition of cyclase activity.

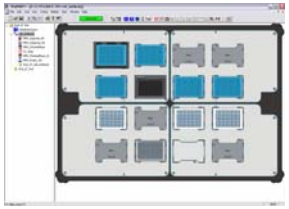
2 Cellular Workstation

3 LANCE cAMP Assay Principle



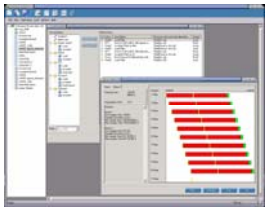
Lance cAMP Assay Principle. Light pulse at 340 nm excites the Europium-chelate of the Eu-SA/b-cAMP tracer. The energy emitted from the Eu-chelate is transferred to the Alexa Fluor© 647 labeled anti-cAMP antibodies bound to the tracer, generating a TR-FRET signal at 665 nm. Residual energy from the Eu-chelate will produce light at 615 nm. cAMP of a sample competes with the tracer for antibody binding sites and causes a signal reduction.

4 WinPREP® for EP3 and POLARA™ Interfaces



WinPREP for EP3

- The EP3 pipetting platform is controlled thought the WinPREP GUI. The EP3 deck has 16 positions that can be fitted with a wide variety of deck accessories and labware.
- The EP3 can be fitted with interchangeable 96 or 384 tip dispense heads and is compatible with 96, 384 or 1536 well plates.
- Seven of the deck positions can be accessed by the robotic arm. The gripper on the EP3 dispense head can perform additional labware movements.
- The liquid handling steps of the procedure tree are built thought an icon-driven interface.



POLARA

- The POLARA suite of software components is used to integrated and control the robotic arm and other instruments that comprise the Cellular Workstation.
- POLARA methods are written that direct the plate movements performed by the arm, and instruct the other components such as the EP3 and EnVision to run liquid handling and plate reading protocols.
- An automated schedule to run 10 plate batches of the LANCE cAMP assay was developed using the POLARA scheduling module. The schedule displayed as a Gantt chart as shown on the left allows visualization the sequence of steps to adjust the schedule to avoid bottlenecks and maximize throughput.

5 Materials and Methods

Assay Protocol Flow

Seed cells in 6 µl of Stimulation Buffer

↓

Add compounds and antibody in 6 µl of Stimulation Buffer

↓

Incubate for 45 to 60 minutes

↓

Add 12 µl of Detection Mix

↓

Incubate for 60 minutes

↓

Read on EnVision

Cell Culture

CHO cells stably transfected with the 5-HT1a receptor were cultured in an incubator at 37° C with 5% CO_2. Cells were dissociated with Cell Dissociation Solution (Catalog Number C-5914, Sigma, Inc.)

Cell growth medium: MEM with 10% FBS, 2 mM L-Glutamine, and 200 µg/ml Geneticin

Assay Reagents and Protocol

The assay was performed following the protocol recommended in the LANCE cAMP 384 kit (Catalog Number AD0263, PerkinElmer Life and Analytical Sciences). The assay tracer, antibody and detection mix are components of the kit.

Stimulation buffer: HBSS 1X containing 5 mM HEPES and 0.1% BSA

Microplates

The assay was performed in white 384-well OptiPlates (Catalog Number 6007299, PerkinElmer Life and Analytical Sciences)

Plate Seeding

Plates were seeded prior to the start of the assay using the FlexDrop™ Precision Reagent Dispense System (PerkinElmer, Inc.). The cells were seeded at a density of 3,000 cells/well and the plates loaded onto the hotel of the CataLyst Express.

Data Analysis

Data was analyzed using GraphPad Prism® software (GraphPad Software, Inc.) Data is plotted as the mean ± 1SD.

6 Results: cAMP Standard Curve

A standard curve was included on each plate of the 10 plate batch to monitor the precision of the liquid handing and the inter-plate variability of the assay. The superposition of the 10 standard curves shown to the right demonstrates the high degree of reproducibility from plate-to-plate throughout the run.

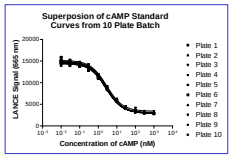


	Plate1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10
IC_{50} (nM)	3.4	3.4	4.1	3.2	3.1	2.8	3.1	2.9	2.9	3.2
Average	3.2	SD	0.37	%CV	11.6					

7 Results: Forskolin Dose/Response Curve

Cells were treated with forskolin to stimulate adenylyl cyclase and a representative dose/response curve plotted to the right. The EC_{50} over the 10 plate batch showed a variability of 11.4 %CV.

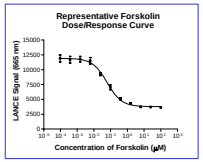


	Plate1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10
EC_{50} (nM)	62	56	55	52	45	48	44	45	51	48
Average	50	SD	5.7	%CV	11.4					

8 Results: Agonist Dose/Response Curve

Agonist response was determined by measuring the 8-OH-DPAT reversal of the forskolin (1 µM, EC_{80}) stimulation of adenylyl cyclase. The EC_{50} of the agonist response showed a variability of 9.1 %CV over the 10 plate batch.

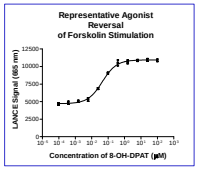


	Plate1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10
EC_{50} (nM)	44	49	50	47	50	41	54	47	50	56
Average	49	SD	4.3	%CV	9.1					

9 Results: Z' Analysis

A Z' analysis of the forskolin response was performed on each plate of the 10 plate batch. 24 wells of basal response were compared to 24 wells of cells stimulated with 100 µM forskolin. The average Z' for the 10 plates was 0.81

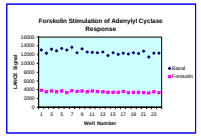


	Plate1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10
Z'	0.76	0.73	0.82	0.80	0.81	0.79	0.87	0.80	0.86	0.82

A Z' analysis was also performed for the agonist reversal of the forskolin response. 24 well of cells stimulated with 1 µM forskolin were compared to 24 wells treated with1µM forskolin and 100 µM 8-OH-DPAT. The average Z' for the 10 plates was 0.71

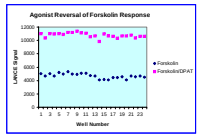


	Plate1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10
Z'	0.67	0.62	0.70	0.69	0.70	0.76	0.73	0.71	0.76	0.72

10 Summary

- The LANCE cAMP assay has been successfully automated on the Cellular Workstation. The assay utilizes a CHO cell line expressing the 5-HT1a receptor, which inhibits the production of cAMP by coupling to adenylyl cyclase through a Gαi-containing G protein.

The Cellular Workstation components used in this assay included:

- Evolution P3 Precision Pipetting Platform
- CataLyst Express Robotic Arm
- EnVision Microplate Reader

The software interfaces controlling the automation process were:

- WinPREP for EP3 for all liquid handling steps
- POLARA for instrument control and scheduling

- The assay format involves two liquid-handling steps, and a 45 minute and a 1 hour incubation step. The lapse time for the assay itself is therefore approximately 2 hours. In order to obtain consistent plate-to-plate signals, the incubation times must be carefully controlled. Processing multiple plates manually is very difficult due to the timing constraints.
- The POLARA software has been used to schedule a 10 plate batch which maintains exact incubation times from plate-to-plate, and staggers the plate processing steps to minimize the total lapse time for the run. The 10 plate batch is completed in approximately 2 hours and 40 minutes. This could not be accomplished manually
- In order to test the precision of the automated assay each plate was formatted with wells to measure:
  - cAMP Standard Curve
  - Forskolin Dose/Response Curve and Z'
  - Agonist Inhibition of Forskolin Stimulation and Z'
- All measures of assay performance were excellent as determined by Z' and %CV values