Use of the JANUS™ Cellular Workstation for the Automation of GPCR Cell-Based Functional Assays Using LANCE™ cAMP Technology

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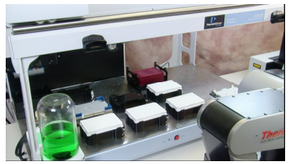
1 Introduction

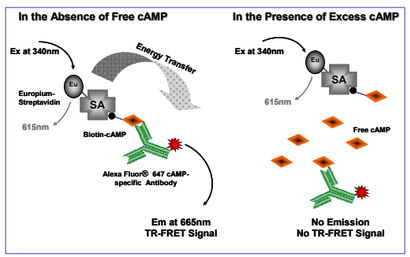
The JANUS™ Cellular Workstation is a fully integrated and automated workstation for performing cell-based assays. The system is comprised of the JANUS™ liquid handling system equipped with a P30 384-channel MDT dispense head , the EnVision™ multilabel plate reader, and a Thermo CRS CataLyst Express robotic arm controlled by POLARA™ scheduling software. Additional integration options may be added to meet specific assay requirements. 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 JANUS 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, and a second cell line expressing the beta-2 adrenergic receptor, a Gαs-coupled receptor that stimulates adenylyl cyclase.

2 JANUS Cellular Workstation



3 LANCE cAMP Assay Principle



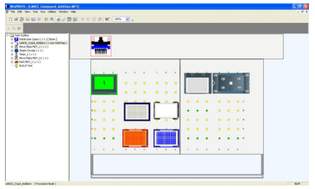
Lance cAMP Assay Principle. Light pulse at 340 nm excites the Europium-chelate of the Eu-SA/b-cAMP tracer. The energy emitted from the Eu-chelate is transferred to the Alexa Fluor© 647 labeled anti-cAMP antibodies bound to the tracer, generating a TR-FRET signal at 665 nm. Residual energy from the Eu-chelate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

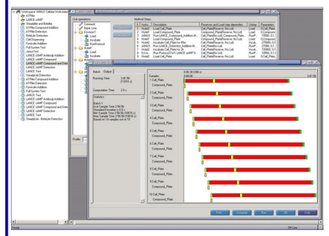
4 WinPREP for JANUS and POLARA Interfaces

WinPREP for JANUS

•The JANUS pipetting system is controlled though WinPREP software. The deck layout (right) used for the LANCE cAMP assay utilizes labware located at the following positions:

MDT-Left [A1]: 1 well Tipwash Tile
MDT-Left [D4]: Variomag Teleshake on an H+P Shaker Plate Support
MDT-Left [D7]: 1 Well low profile reagent reservoir containing the detection mixture
MDT-Left [G6]: 384 Well OptiPlate containing the antibody solution or a 384 well ProxiPlate containing the samples or standard
MDT-Left [G7]: 384 Well OptiPlate containing the cell suspension
MDT-Right [A1]: P30 – 384 Tip box on a TipLoad support tile





POLARA

•The POLARA software is used to control the robotic arm for plate movement between the instruments of the Workstation and schedule the timing of the assay steps. Two POLARA schedules are used to run the assay.

•The first transfers plates containing the cell suspension to the JANUS for addition of the antibody solution.

• The second (left) transfers sets of compound and assay plates to the JANUS for addition of the compounds to the assay plates in a single pipetting step using the 384-channel dispense head. The plates are then transferred back to the hotels for a 45 minute incubation. The assay plates are then transferred to the JANUS for addition of the detection mix. Following a further 2 hour incubation on the hotel the plates are transferred to the EnVision for reading.

5 Materials and Methods

Assay Protocol Flow

Seed cells in 6 µl of Stimulation Buffer
↓
Add antibody in 3 µl of Stimulation Buffer
↓
Add compounds in 3 µl of Stimulation Buffer
↓
Incubate for 45 to 60 minutes
↓
Add 12 µl of Detection Mix
↓
Incubate for 2 Hours
↓
Read on the EnVision

Cell Culture
CHO cells stably transfected with either the 5-HT1a receptor or the beta-2 adrenergic 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, Inc.).

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.

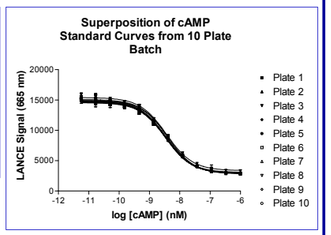


	Plate 1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10
IC_{50} (nM)	1.9	2.1	2.1	1.8	2.0	1.9	1.9	2.2	1.9	1.8
Average	2.0	SD	0.14	%CV	6.9					

7 Results: Gs Agonist Dose-Response Curve

Cells expressing the beta-2 adrenergic receptor were treated with epinephrine 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 3.2 %CV.

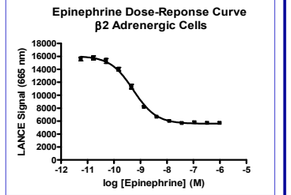


	Plate1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10
EC_{50} (nM)	0.46	0.43	0.45	0.44	0.43	0.43	0.42	0.42	0.43	0.43
Average	0.43	SD	0.01	%CV	3.2					

8 Results: Gi Agonist Dose-Response Curve

Agonist response in cells expressing the 5-HT1a receptor 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 4.1 %CV over the 10 plate batch.

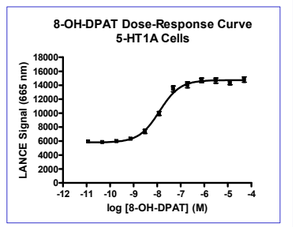


	Plate1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10
EC_{50} (nM)	12	11	11	11	11	11	11	12	12	11
Average	11	SD	0.46	%CV	4.1					

9 Results: Z' Analysis

A Z' analysis of the 8-OH-DPAT response was performed on each plate of a 10 plate batch using cells expressing the 5-HT1a receptor. 32 wells of basal response were compared to 32 wells of cells stimulated with 100 µM forskolin. The average Z' for the 10 plates was 0.83

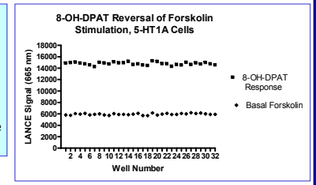


	Plate1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10
Z'	0.83	0.81	0.83	0.85	0.84	0.84	0.81	0.84	0.82	0.81

A Z' analysis was also performed for the epinephrine response of a 10 plate batch of cells expressing the beta-2 receptor. 32 wells of cells stimulated with 1 µM epinephrine were compared to 24 wells of basal response. The average Z' for the 10 plates was 0.88.

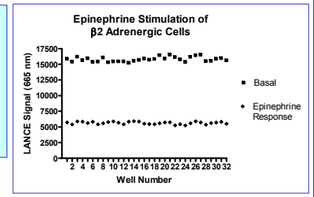


	Plate1	Plate 2	Plate 3	Plate 4	Plate 5	Plate 6	Plate 7	Plate 8	Plate 9	Plate 10
Z'	0.90	0.90	0.87	0.87	0.86	0.89	0.88	0.89	0.88	0.87

10 Summary

- The LANCE cAMP assay has been successfully automated using the JANUS Cellular Workstation. The assay utilizes CHO cell lines expressing either the 5-HT1a receptor or the beta-2 adrenergic receptor.
- The Workstation components used in this assay include:
  - JANUS MDT Liquid Handling System
  - CataLyst Express Robotic Arm
  - EnVision Microplate Reader
- The assay format involves three liquid handling steps, a 45 minute and a 2 hour incubation step, and plate reading. The lapse time for the assay is therefore approximately 4 hours. In order to obtain consistent plate-to-plate signals, the incubation times must be carefully controlled.
- The POLARA software has been used to schedule 10 plate batches which maintain exact incubation times from plate-to-plate, and staggers the plate processing steps to minimize the total lapse time for the run.
- In order to test the precision of the automated assay each plate was formatted with wells to measure:
  - cAMP Standard Curve
  - Gs and Gi Agonist Dose-Response
  - Gs and Gi Agonist Z'
- All measures of assay performance were excellent as determined by Z' and %CV values.

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