Use of the JANUS™ Cellular Workstation for the Automation of GPCR Cell-Based Functional Assays

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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 dispenser head, the Envision™ multilabel plate reader, and a Thermo CROS Calypso Express robotic arm controlled by POLARIA™ scheduling software. Additional integration options may be added to meet specific assay requirements. This workstation is a walkaway, 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) immun assay designed to measure cAMP produced upon stimulation 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 Gi protein that leads to the inhibition of cyclic activity, and a second cell line expressing the beta-2 adrenergic receptor, a Gs-coupled receptor that stimulates adenylyl cyclase.

2 JANUS Cellular Workstation

WinPREP for JANUS and POLARIA Interfaces

WinPREP for JANUS

The JANUS® WinPREP scheduling system is controlled through WinPREP software. The deck file for the JANUS® WinPREP scheduling system utilizes laboratory locators at the MDT-Lift and Polarisate stations. 

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 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 POLARIA Interfaces

WinPREP for JANUS

A standard curve was included on the LANCE cAMP plate 1. A Superposition of cAMP Stimulation, 5-HT1A Cells 1.82 2.0 1.8 2.1 2.1 2.0 1.9 1.9 2.2 1.9 1.8

5 Materials and Methods

Assay Protocol Flow

Cell Culture

Cells were stably transfected with either the 5-HT1A receptor or the beta-2 adrenergic receptor were cultured in an incubator at 37°C with 5% CO2. Cells were dissociated with Cell Dissociation Solution (Catalog Number C-05141, Sigma, Inc.). Cell growth medium: MEM with 10% FBS, 2 mM L-Glutamine, and 200 µg/ml G418.

Assay Reagents and Protocol

A protocol was performed following the protocol recommended in the LANCE cAMP 384 kit (Catalog Number AD0263, PerkinElmer Life and Analytical Sciences). The assay reagent, tracer and detection mix are components of the LANCE cAMP 384 kit stimulation buffer. HBSS TX containing 5 mM HEPES and 0.1% BSA.

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 handling and the inter-plate variability of the assay. The superposition of the standard curves shown to the right demonstrates the high degree of reproducibility from plate-to-plate throughout the run.

7 Results: Gs Agonist Dose-Response Curve

Cells expressing the beta-2 adrenergic receptor were treated with epinephrine to stimulate adenyl cyclase and a representative dose-response curve utilized to the right. The EC50 of the 12 plate batch showed a variability of 2.46 ± 0.49.

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) stimulation of adenylyl cyclase. The EC50 of the agonist response showed a variability of 0.11 ± 0.11 over the 10 plate batch.

9 Results: Z' Analysis

Z’ analysis of the 8-OH-DPAT response was performed on each plate of the 10 plate batch of cells expressing the 5-HT1A receptor. 32 wells of basal response were compared to 32 wells of cells treated with 100 µg/ml forskolin. The average Z’ for the 10 plates was 0.83 ± 0.26.

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 incubation. The assay plates are then transferred to the EnVision for addition of the detection mix. Following a further 2 hour incubation on the plate, the plates are transferred to the EnVision for reading. The assay was performed on a 10 plate batch of cells expressing the 5-HT1A receptor. 32 wells of basal response were compared to 32 wells of cells treated with 100 µg/ml forskolin. The average Z’ for the 10 plates was 0.83 ± 0.26.