eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ ChemiScreen™ Chem-6 Wild-type Parental Control Frozen Cells

CATALOG NUMBER: HTSCHEM-6RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Eurofins Discovery Services' Chem-6 Parental Control Frozen Cells are designed for use as a negative control alongside our GPCR-expressing Ready-to-Assay Frozen Cells in the Chem-6, Chem-7, and Chem-8 backgrounds. Chem-6 cells lack endogenous expression of most GPCRs, although they endogenously express trypsin and thrombin receptors that may be used to confirm responsiveness of the cells in calcium assays. The Chem-6 cell line is adherent to tissue culture-treated surfaces and contains high levels of the promiscuous G protein Gα15 to couple many classes of GPCRs to the calcium signaling pathway.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

APPLICATIONS

Calcium Flux Assays ; cAMP accumulation

ASSAY SETUP

Fluorescence

Table 1. Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Fluorescence

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Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	95 µl (50 µl for 384-well)
Dispense Speed	50 µl/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 2. Fluorescence Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	Millipore Sigma: H3537
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712

Assay Protocol – Fluorescence

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.
8. Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*
9. Remove plate from incubator and quickly invert plate on an absorbent pad and blot to remove all Media Component.
10. Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.
11. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
12. Create protocol for ligand addition. Please refer to FLIPR^{TETRA}® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.
13. After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

Chem-6, an adherent Chinese Hamster Ovary cell line expressing endogenous Gα15 protein.

RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-6	ChemiScreen™ Chem-6 Wild-Type Parental Control

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User Agreement (Label License)

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