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# PRODUCT DATASHEET

## ChemiScreen™ $mGLU_2$ Metabotropic Glutamate Receptor Stable Cell Line

## CATALOG NUMBER: HTS146C

**CONTENTS**: 2 vials of mycoplasma-free cells, 1 mL per vial.

**STORAGE**: Vials are to be stored in liquid $N_2$.

## BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

Glutamate is a main excitatory neurotransmitter in the central nervous system, and it plays a role in learning, memory and neurotoxicity. The biological actions of glutamate are mediated by ionotropic and metabotropic glutamate receptors, which are ion channels and GPCRs respectively. Metabotropic glutamate receptors (mGluRs) are members of the class 3 G-protein coupled receptor family, which are characterized by a large extracellular domain. They are further classified into group I, II, and III mGluRs on the basis of their sequence identity, pharmacology, and signal transduction mechanism. Group I ($mGlu_1$ and $mGlu_5$) couple to the phospholipase C pathway through $G_{\alpha q}$, whereas group II ($mGlu_2$ and $mGlu_3$) and group III ($mGlu_4$, $mGlu_6$, $mGlu_7$, and $mGlu_8$) negatively couple to the adenylyl cyclase pathway though $G_{\alpha i}$ (Conn and Pin, 1997). Agonists of the Group II metabotropic glutamate receptors, $mGlu_2$ and $mGlu_3$, display efficacy in animal models of anxiety and psychosis. A key role for $mGlu_2$ in mediating these effects is indicated by the observation that selective allosteric potentiator of $mGlu_2$ also retains antipsychotic-like activities in mice (Galici *et al.*, 2005). In addition, $mGlu_{2/3}$ agonists display analgesic activity in animal models (Jones *et al.*, 2005). The cloned human $mGlu_2$-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant $mGlu_2$ expression on the cell surface and contains high levels of the promiscuous G protein $G_{\alpha 15}$ to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and allosteric modulators of $mGlu_2$.

## USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

## 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.

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## APPLICATIONS

Calcium Flux Fluorescence Assay

### APPLICATION DATA

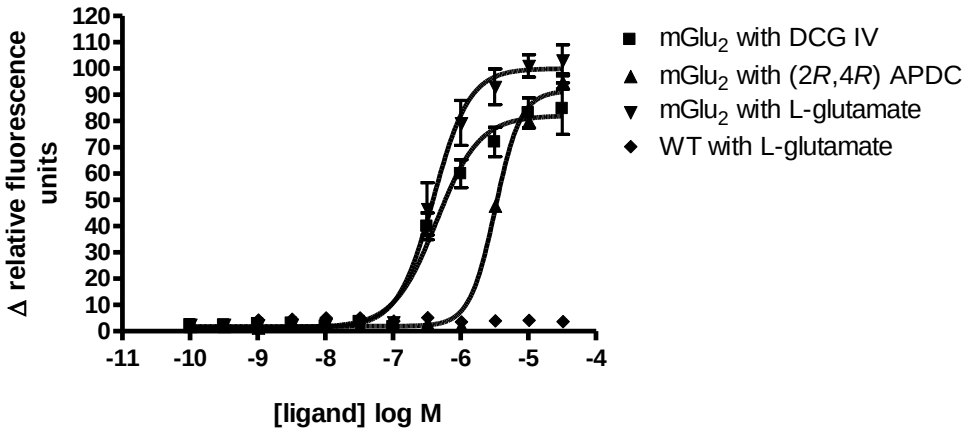


Figure 1. Representative data for activation of the $mGLU_2$ receptor stably expressed in Chem-1 cells induced by DCG IV using a fluorescent calcium flux assay. $mGLU_2$–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR$^{TETRA}$® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 8,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. $EC_{50}$ value of $mGLU_2$-expressing Chem-1 cells.

| LIGAND | ASSAY | POTENCY $EC_{50}$ (nM) | REFERENCE |
|---|---|---|---|
| **DCG IV** | Calcium Flux - Fluorescence | 0.51 | Eurofins Internal Data |

* The cell line was tested and found to have equivalent $EC_{50}$ and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence.

## CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

| Description | Component | Concentration | Supplier and Product Number |
|---|---|---|---|
| **Basal Medium** | DMEM high glucose Medium (4.5g/L) | - | Hyclone: SH30022 |
| | Fetal Bovine Serum (FBS) | 10% | Hyclone: SH30070.03 |
| | Non-Essential Amino Acids (NEAA) | 1X | Hyclone: SH30238.01 |
| | HEPES | 1X | EMD Millipore: TMS-003-C |
| **Selection Medium** | Basal Medium (see above) | - | |
| | Geneticin (G418) | 250 µg/ml | Invivogen: ant-gn-5 |
| **Dissociation** | Sterile PBS | - | Hyclone: SH30028.03 |
| | 0.25% Trypsin-EDTA | - | Hyclone: SH30042.01 |
| **CryoMedium** | Basal Medium (see above) | 40% | |
| | Fetal Bovine Serum (FBS) | 50% | Hyclone: SH30070.03 |
| | Dimethyl Sulfoxide (DMSO) | 10% | Sigma: D2650 |

## Cell Handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% $CO_2$.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, At this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min*. Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

| Flask Size ($cm^2$) | Volume (mL) | Total Cell Number ($x10^6$) | Growth Period (hrs) |
|---|---|---|---|
| **T75** | 15 | 5.0 | 24 |
| **T75** | 15 | 2.0 | 48 |
| **T75** | 15 | 0.45 | 72 |

# ASSAY SETUP

## Fluorescence

Table 4. Settings for FLIPR$^{TETRA}$® with ICCD camera option

| Option | Setting |
|---|---|
| Read Mode | Fluorescence |
| 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 5. Assay Materials (Not provided)

| Description | Supplier and Product Number |
|---|---|
| HBSS | Invitrogen: 14025 |
| HEPES 1M Stock | EMD Millipore: TMS-003-C |
| Probenicid | Sigma: P8761 |
| Quest Fluo-8™, AM | AAT Bioquest: 21080 |
| DCG IV ligand | Tocris: 1827 |
| Non-Binding 96/384 well Plates (for ligand prep) | Corning: 3605/ 3574 |
| Black (clear Bottom) cell assay plates | Corning: 3904/ 3712 |
| Coelenterazine-h (250µg). Prepare to 10mM | Promega: S2011 |

## Assay Protocol – Fluorescence

1. Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability
2. Centrifuge the cell suspension at 190 x g for six min
3. Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve $5x10^5$cells/ml *(i.e, if collected 5e6 TC,* $^{5e6/}_{5e5/ml}$ *=10 mL volume)*
4. Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*
5. Move assay plate to a humidified 37ºC 5% $CO_2$ incubator for 18-24 h.
6. 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*
7. Remove medium from assay plate and wash 1X with Assay Buffer.
8. 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.
9. Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.
10. 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.
11. 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-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

## EXOGENOUS GENE EXPRESSION

Human $mGLU_2$ cDNA (Accession Number: NM_000839; see CODING SEQUENCE below) and promiscuous G protein are expressed in a bicistronic vector

## CODING SEQUENCE

```
   1 ATGGGATCGCTGCTTGCGCTCCTGGCACTGCTGCTGCTGTGGGGTGCTGTGGCTGAGGGCCCAGCCAAGAAGGTGCTGACCCTGGAGGGA
   1  M  G  S  L  L  A  L  L  A  L  L  L  L  W  G  A  V  A  E  G  P  A  K  K  V  L  T  L  E  G

  91 GACTTGGTGCTGGGTGGGCTGTTCCCAGTGCACCAGAAGGGCGGCCCAGCAGAGGACTGTGGTCCTGTCAATGAGCACCGTGGCATCCAG
  31  D  L  V  L  G  G  L  F  P  V  H  Q  K  G  G  P  A  E  D  C  G  P  V  N  E  H  R  G  I  Q

 181 CGCCTGGAGGCCATGCTTTTTGCACTGGACCGCATCAACCGTGACCCGCACCTGCTGCCTGGCGTGCGCCTGGGTGCACACATCCTCGAC
  61  R  L  E  A  M  L  F  A  L  D  R  I  N  R  D  P  H  L  L  P  G  V  R  L  G  A  H  I  L  D

 271 AGTTGCTCCAAGGACACACATGCGCTGGAGCAGGCACTGGACTTTGTGCGTGCCTCACTCAGCCGTGGTGCTGATGGCTCACGCCACATC
  91  S  C  S  K  D  T  H  A  L  E  Q  A  L  D  F  V  R  A  S  L  S  R  G  A  D  G  S  R  H  I

 361 TGCCCCGACGGCTCTTATGCGACCCATGGTGATGCTCCCACTGCCATCACTGGTGTTATTGGCGGTTCCTACAGTGATGTCTCCATCCAG
 121  C  P  D  G  S  Y  A  T  H  G  D  A  P  T  A  I  T  G  V  I  G  G  S  Y  S  D  V  S  I  Q

 451 GTGGCCAACCTCTTGAGGCTATTTCAGATCCCACAGATTAGCTACGCCTCTACCAGTGCCAAGCTGAGTGACAAGTCCCGCTATGACTAC
 151  V  A  N  L  L  R  L  F  Q  I  P  Q  I  S  Y  A  S  T  S  A  K  L  S  D  K  S  R  Y  D  Y

 541 TTTGCCCGCACAGTGCCTCCTGACTTCTTCCAAGCCAAGGCCATGGCTGAGATTCTCCGCTTCTTCAACTGGACCTATGTGTCCACTGTG
 181  F  A  R  T  V  P  P  D  F  F  Q  A  K  A  M  A  E  I  L  R  F  F  N  W  T  Y  V  S  T  V

 631 GCGTCTGAGGGCGACTATGGCGAGACAGGCATTGAGGCCTTTGAGCTAGAGGCTCGTGCCCGCAACATCTGTGTGGCCACCTCGGAGAAA
 211  A  S  E  G  D  Y  G  E  T  G  I  E  A  F  E  L  E  A  R  A  R  N  I  C  V  A  T  S  E  K

 721 GTGGGCCGTGCCATGAGCCGCGCGGCCTTTGAGGGTGTGGTGCGAGCCCTGCTGCAGAAGCCCAGTGCCCGCGTGGCTGTCCTGTTCACC
 241  V  G  R  A  M  S  R  A  A  F  E  G  V  V  R  A  L  L  Q  K  P  S  A  R  V  A  V  L  F  T

 811 CGTTCTGAGGATGCCCGGGAGCTGCTTGCTGCCAGCCAGCGCCTCAATGCCAGCTTCACCTGGGTGGCCAGTGATGGTTGGGGGGCCCTG
 271  R  S  E  D  A  R  E  L  L  A  A  S  Q  R  L  N  A  S  F  T  W  V  A  S  D  G  W  G  A  L

 901 GAGAGTGTGGTGGCAGGCAGTGAGGGGGCTGCTGAGGGTGCTATCACCATCGAGCTGGCCTCCTACCCCATCAGTGACTTTGCCTCCTAC
 301  E  S  V  V  A  G  S  E  G  A  A  E  G  A  I  T  I  E  L  A  S  Y  P  I  S  D  F  A  S  Y

 991 TTCCAGAGCCTGGACCCTTGGAACAACAGCCGGAACCCCTGGTTCCGTGAATTCTGGGAGCAGAGGTTCCGCTGCAGCTTCCGGCAGCGA
 331  F  Q  S  L  D  P  W  N  N  S  R  N  P  W  F  R  E  F  W  E  Q  R  F  R  C  S  F  R  Q  R

1081 GACTGCGCAGCCCACTCTCTCCGGGCTGTGCCCTTTGAGCAGGAGTCCAAGATCATGTTTGTGGTCAATGCAGTGTACGCCATGGCCCAT
 361  D  C  A  A  H  S  L  R  A  V  P  F  E  Q  E  S  K  I  M  F  V  V  N  A  V  Y  A  M  A  H

1171 GCGCTCCACAACATGCACCGTGCCCTCTGCCCCAACACCACCCGGCTCTGTGACGCGATGCGGCCAGTTAACGGGCGCCGCCTCTACAAG
 391  A  L  H  N  M  H  R  A  L  C  P  N  T  T  R  L  C  D  A  M  R  P  V  N  G  R  R  L  Y  K

1261 GACTTTGTGCTCAACGTCAAGTTTGATGCCCCCTTTCGCCCAGCTGACACCCACAATGAGGTCCGCTTTGACCGCTTTGGTGATGGTATT
 421  D  F  V  L  N  V  K  F  D  A  P  F  R  P  A  D  T  H  N  E  V  R  F  D  R  F  G  D  G  I

1351 GGCCGCTACAACATCTTCACCTATCTGCGTGCAGGCAGTGGGCGCTATCGCTACCAGAAGGTGGGCTACTGGGCAGAAGGCTTGACTCTG
 451  G  R  Y  N  I  F  T  Y  L  R  A  G  S  G  R  Y  R  Y  Q  K  V  G  Y  W  A  E  G  L  T  L

1441 GACACCAGCCTCATCCCATGGGCCTCACCCTCAGCCGGCCCCCTGCCCGCCTCTCGCTGCAGTGAGCCCTGCCTCCAGAATGAGGTGAAG
 481  D  T  S  L  I  P  W  A  S  P  S  A  G  P  L  P  A  S  R  C  S  E  P  C  L  Q  N  E  V  K

1531 AGTGTGCAGCCGGGCGAAGTCTGCTGCTGGCTCTGCATTCCGTGCCAGCCCTATGAGTACCGATTGGACGAATTCACTTGCGCTGATTGT
 511  S  V  Q  P  G  E  V  C  C  W  L  C  I  P  C  Q  P  Y  E  Y  R  L  D  E  F  T  C  A  D  C

1621 GGCCTGGGCTACTGGCCCAATGCCAGCCTGACTGGCTGCTTCGAACTGCCCCAGGAGTACATCCGCTGGGGCGATGCCTGGGCTGTGGGA
 541  G  L  G  Y  W  P  N  A  S  L  T  G  C  F  E  L  P  Q  E  Y  I  R  W  G  D  A  W  A  V  G

1711 CCTGTCACCATCGCCTGCCTCGGTGCCCTGGCCACCCTCTTTGTGCTGGGTGTCTTTGTGCGGCACAATGCCACACCAGTGGTCAAGGCC
 571  P  V  T  I  A  C  L  G  A  L  A  T  L  F  V  L  G  V  F  V  R  H  N  A  T  P  V  V  K  A

1801 TCAGGTCGGGAGCTCTGCTACATCCTGCTGGGTGGTGTCTTCCTCTGCTACTGCATGACCTTCATCTTCATTGCCAAGCCATCCACGGCA
 601  S  G  R  E  L  C  Y  I  L  L  G  G  V  F  L  C  Y  C  M  T  F  I  F  I  A  K  P  S  T  A

1891 GTGTGTACCTTACGGCGTCTTGGTTTGGGCACTGCCTTCTCTGTCTGCTACTCAGCCCTGCTCACCAAGACCAACCGCATTGCACGCATC
 631  V  C  T  L  R  R  L  G  L  G  T  A  F  S  V  C  Y  S  A  L  L  T  K  T  N  R  I  A  R  I

1981 TTCGGTGGGGCCCGGGAGGGTGCCCAGCGGCCACGCTTCATCAGTCCTGCCTCACAGGTGGCCATCTGCCTGGCACTTATCTCGGGCCAG
 661  F  G  G  A  R  E  G  A  Q  R  P  R  F  I  S  P  A  S  Q  V  A  I  C  L  A  L  I  S  G  Q

2071 CTGCTCATCGTGGTCGCCTGGCTGGTGGTGGAGGCACCGGGCACAGGCAAGGAGACAGCCCCCGAACGGCGGGAGGTGGTGACACTGCGC
 691  L  L  I  V  V  A  W  L  V  V  E  A  P  G  T  G  K  E  T  A  P  E  R  R  E  V  V  T  L  R

2161 TGCAACCACCGCGATGCAAGTATGTTGGGCTCGCTGGCCTACAATGTGCTCCTCATCGCGCTCTGCACGCTTTATGCCTTCAAGACTCGC
 721  C  N  H  R  D  A  S  M  L  G  S  L  A  Y  N  V  L  L  I  A  L  C  T  L  Y  A  F  K  T  R

2251 AAGTGCCCCGAAAACTTCAACGAGGCCAAGTTCATTGGCTTCACCATGTACACCACCTGCATCATCTGGCTGGCATTCCTGCCCATCTTC
```

```
 751   K  C  P  E  N  F  N  E  A  K  F  I  G  F  T  M  Y  T  T  C  I  I  W  L  A  F  L  P  I  F

2341  TATGTCACCTCCAGTGACTACCGGGTACAGACCACCACCATGTGCGTGTCAGTCAGCCTCAGCGGCTCCGTGGTGCTTGGCTGCCTCTTT
 781   Y  V  T  S  S  D  Y  R  V  Q  T  T  T  M  C  V  S  V  S  L  S  G  S  V  V  L  G  C  L  F

2431  GCGCCCAAGCTGCACATCATCCTCTTCCAGCCGCAGAAGAACGTGGTTAGCCACCGGGCACCCACCAGCCGCTTTGGCAGTGCTGCTGCC
 811   A  P  K  L  H  I  I  L  F  Q  P  Q  K  N  V  V  S  H  R  A  P  T  S  R  F  G  S  A  A  A

2521  AGGGCCAGCTCCAGCCTTGGCCAAGGGTCTGGCTCCCAGTTTGTCCCCACTGTTTGCAATGGCCGTGAGGTGGTGGACTCGACAACGTCA
 841   R  A  S  S  S  L  G  Q  G  S  G  S  Q  F  V  P  T  V  C  N  G  R  E  V  V  D  S  T  T  S

2611  TCGCTT TGA
 871   S  L  Stp
```

## RELATED PRODUCTS

| Product Number | Description |
|---|---|
| **HTSCHEM-1** | ChemiScreen™ Chem-1 Parental Cell Line (control cells) |
| **HTS146M** | ChemiScreen™ $mGLU_2$ Metabotropic Glutamate Receptor Membrane Prep |

## REFERENCES


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3. Galici R *et al.* (2006) Biphenyl-indanone A, a positive allosteric modulator of the metabotropic glutamate receptor subtype 2, has antipsychotic- and anxiolytic-like effects in mice. *J. Pharmacol. Exp. Ther.* 318: 173-185.
4. Jones CK *et al.* (2005) Analgesic effects of the selective group II (mGlu2/3) metabotropic glutamate receptor agonists LY379268 and LY389795 in persistent and inflammatory pain models after acute and repeated dosing. *Neuropharmacology* 49 Suppl 1:206-18.
5. Kowal D et al. (2003) Functional calcium coupling with the human metabotropic glutamate receptor subtypes 2 and 4 by stable co-expression with a calcium pathway facilitating G-protein chimera in Chinese hamster ovary cells. *Biochem. Pharmacol.* 66: 785-790.
6. Schoepp DD *et al*. (1997) The novel metabotropic glutamate receptor agonist 2*R*,4*R*-APDC potentiates stimulation of phosphoinositide hydrolysis in the rat hippocampus by 3,5-dihydroxyphenylglycine: evidence for a synergistic interaction between group 1 and group 2 receptors. *Neuropharmacology* 35: 1661-1672.