

PRODUCT DATASHEET

Ready-to-Assay™ KiSS1 KiSS1-Derived Peptide Receptor Frozen Cells

CATALOG NUMBER: HTS032RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N₂. Media Component at 4°C (-20°C for prolonged storage).

BACKGROUND

Ready-to-Assay™ GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following overnight recovery, assays for calcium response.

KiSS1 (also known as GPR54 and AXOR12) is a Gq-coupled GPCR that binds to a series of peptides, termed kisspeptins or metastin, derived from the KiSS-1 gene (Kotani *et al.*, 2001; Muir *et al.*, 2001). The KiSS-1 gene inhibits metastatic activity of melanoma and other tumor cell lines, and clinical evidence supports a role for KiSS-1 and GPR54 in inhibition of metastasis in human cancer (Harms *et al.*, 2003). Kisspeptins and GPR54 also play a central role in hypothalamic regulation of puberty, by directly governing the release of gonadotropin-releasing hormone from the hypothalamus (Messenger *et al.*, 2005; Shahab *et al.*, 2005). In addition, mutations in GPR54 in mice and humans result in hypogonadotropic hypogonadism (Colledge, 2004; Seminara *et al.*, 2004). Cloned human KiSS1-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant KiSS1 expression on the cell surface for functional detection via the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists, and modulators at KiSS1.

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

APPLICATION DATA

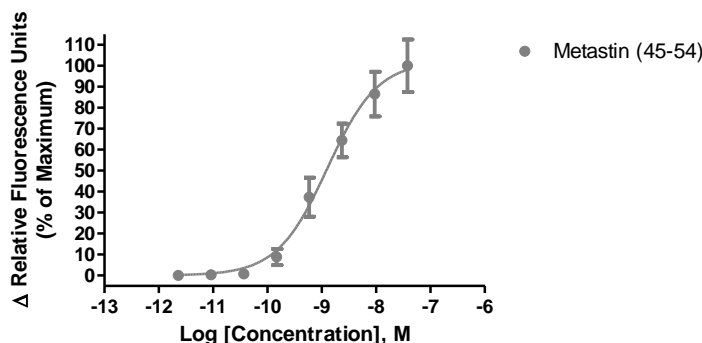


Figure 1. Representative data for activation of KiSS1 receptor. Calcium flux in KiSS1–expressing Chem-1 cell line induced by Metastin (45-54). KiSS1–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA}. Maximal fluorescence signal obtained in this experiment was 2,600 RLU (Relative Light Units).

Table 1. EC₅₀ value of KiSS1-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Metastin (45-54)	Calcium Flux	1.3	Eurofins Internal Data

ASSAY SETUP

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 37°C 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. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37°C 5% CO₂ incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and wash sufficiently with Hank's Balanced Salt Solution (HBSS) supplemented with 20mM HEPES, 2.5mM Probenecid at pH 7.4 to remove all trace of Media Component.

10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca²⁺ dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca²⁺ dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca²⁺ dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm (FLIPR^{TETRA}) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR^{TETRA}) or emission filter for Ca²⁺ dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.
12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH30268.02
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenecid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Metastin (45-54) ligand	Calbiochem: 445888
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

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	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein

EXONGENOUS GENE EXPRESSION

Human KISSR1 cDNA (Accession Number: NM_032551; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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                                     ATG CAC ACC GTG GCT ACG
                                     M  H  T  V  A  T
ACC GGA CCC AAC GCG TCC TGG GGG GCA CCG GCC AAC GCC TCC GGC TGC CCG GGC TGT GGC
T  G  P  N  A  S  W  G  A  P  A  N  A  S  G  C  P  G  C  G
GCC AAC GCC TCG GAC GGC CCA GTC CCT TCG CCG CGG GCC GTG GAC GCC TGG CTC GTG CCG
A  N  A  S  D  G  P  V  P  S  P  R  A  V  D  A  W  L  V  P
CTC TTC TTC GCG GCG CTG ATG CTG CTG GGC CTG GTG GGG AAC TCG CTG GTC ATC TAC GTC
L  F  F  A  A  L  M  L  L  G  L  V  G  N  S  L  V  I  Y  V
ATC TGC CGC CAC AAG CCG ATG CGG ACC GTG ACC AAC TTC TAC ATC GCC AAC CTG GCG GCC
I  C  R  H  K  P  M  R  T  V  T  N  F  Y  I  A  N  L  A  A
ACG GAC GTG ACC TTC CTC CTG TGC TGC GTC CCC TTC ACG GCC CTG CTG TAC CCG CTG CCC
T  D  V  T  F  L  L  C  C  V  P  F  T  A  L  L  Y  P  L  P
GGC TGG GTG CTG GCG GAC TTC ATG TGC AAG TTC GTC AAC TAC ATC CAG CAG GTC TCG GTG
G  W  V  L  G  D  F  M  C  K  F  V  N  Y  I  Q  Q  V  S  V
CAG GCC ACG TGT GCC ACT CTG ACC GCC ATG AGT GTG GAC CGC TGG TAC GTG ACG GTG TTC
Q  A  T  C  A  T  L  T  A  M  S  V  D  R  W  Y  V  T  V  F
CCG TTG CGC GCC CTG CAC CGC CGC ATG P  R  L  A  L  A  V  S  L  S  I
P  L  R  R  A  C  H  R  R  C  P  R  L  A  L  A  V  S  L  S  I
TGG GTA GGC TCT GCG GCG GTG TCT GCG CCG GTG CTC GCC CTG CAC CGC CTG TCA CCC GGG
W  V  A  G  S  A  A  V  S  A  P  V  L  A  L  H  R  L  S  P  G
CCG CGC GCC TAC TGC AGT GAG GCC TTC CCC AGC CGC GCC CTG GAG CGC GCC TTC GCA CTG
P  R  A  Y  C  S  E  A  F  P  S  R  A  L  E  R  A  F  A  L
TAC AAC CTG CTG GCG CTG TAC CTG CTG CCG CTG CTC GCC ACC TGC GCC TGC TAT GCG GCC
Y  N  L  L  A  L  Y  L  L  P  L  L  A  T  C  A  C  Y  A  A
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M  L  R  H  L  G  R  V  A  V  R  P  A  P  A  D  S  A  L  Q
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G  Q  V  L  A  E  R  A  G  A  V  R  A  K  V  S  R  L  V  A
GCC GTG GTC CTG CTC TTC GCC GCC TGC TGG GGC CCC ATC CAG CTG TTC CTG GTG CTG CAG
A  V  V  L  L  F  A  A  C  W  G  P  I  Q  L  F  L  V  L  Q
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A  L  G  P  A  G  S  W  H  P  R  S  Y  A  A  Y  A  L  K  T
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G  S  H  P  A  P  A  R  A  Q  K  P  G  S  S  G  L  A  A  R
GGG CTG TGC GTC CTG GGG GAG GAC AAC GCC CCT CTC TGA
G  L  C  V  L  G  E  D  N  A  P  L  Stp

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RELATED PRODUCTS

PRODUCT NUMBER

DESCRIPTION

HTSCHEM-1RTA

Ready-to-Assay™ Chem-1 host frozen cells (control cells)

REFERENCES

1. Colledge WH (2004) GPR54 and puberty. *Trends Endocrinol. Metab.* 15: 448-53.
2. Harms JF *et al.* (2003) KISS1 metastasis suppression and emergent pathways. *Clin. Exp. Metastasis* 20: 11-8.
3. Kotani M *et al.* (2001) The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J. Biol. Chem.* 276: 34631-6.
4. Messenger S *et al.* (2005) Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. *Proc. Natl. Acad. Sci. USA* 102: 1761-6.
5. Muir AI *et al.* (2001) AXOR12, a novel human G protein-coupled receptor, activated by the peptide KiSS-1. *J. Biol. Chem.* 276: 28969-75.
6. Shahab M *et al.* (2005) Increased hypothalamic GPR54 signaling: a potential mechanism for initiation of puberty in primates. *Proc. Natl. Acad. Sci. USA* 102: 2129-34.

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