

## PRODUCT DATASHEET

**Ready-to-Assay™ APJ Apelin Receptor  
Frozen Cells****CATALOG NUMBER: HTS068RTA****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 N<sub>2</sub>. 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 over night recovery, assays for calcium response.

Apelin peptides have been discovered to be a family of peptides of different sizes that is derived from the N-terminus of a 77 amino acid precursor peptide (preproapelin) (Hosoya et al., 2000). Apelin receptor (APJ) is a G protein-coupled receptor that is activated by several apelin fragments, which results in inhibition of cAMP production (Habata et al., 1999). APJ and apelin peptides have been found to be involved in the regulation of cardiovascular function (Katugampola et al., 2001) and fluid homeostasis (Reaux et al., 2001). Broad roles of apelin system has been established in lowering blood pressure, as a potent cardiac inotrope, in modulating pituitary hormone release and food and water intake, in stress activation, and as a novel adipokine that is excreted from fat cells and regulates insulin (Lee et al., 2006). Cloned human APJ-expressing cell line is made in the Chem-5 host, which supports high levels of recombinant APJ expression on the cell surface and contains optimized levels of a recombinant promiscuous G protein to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at APJ

**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

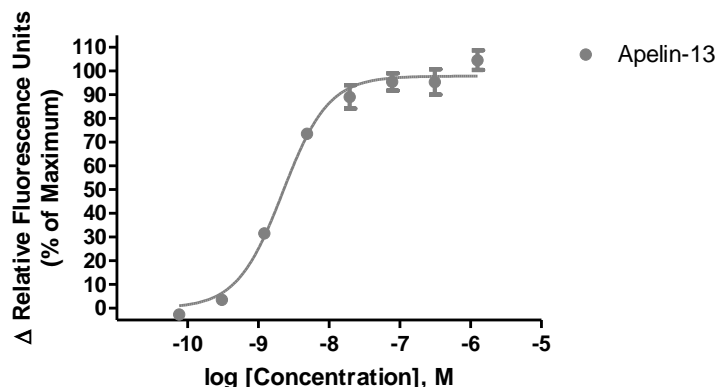


Figure 1. Representative data for activation of APJ receptor. Calcium flux in APJ-expressing Chem-5 cell line induced by Apelin-13 APJ-expressing Chem-5 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPRETETRA plus ICCD. Maximal fluorescence signal obtained in this experiment was 22,000 RLU (Relative Light Units).

Table 1. EC<sub>50</sub> values of APJ -expressing Chem-5 cells with values described in the literature.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Apelin-13	Calcium Flux	2.5	Eurofins Internal Data
Apelin-13	Calcium Flux	22.4	Medhurst et al., 2003

## 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% CO<sub>2</sub> 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) Ca<sup>2+</sup> dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once

dissolved place 10  $\mu$ L of Fluo-8 NW  $\text{Ca}^{2+}$  dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid p buffer and apply to assay microplate ( $\text{Ca}^{2+}$  dye at 10  $\mu$ L /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<sup>TETRA</sup>) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm (FLIPR<sup>TETRA</sup>) or 520 nm (FLIPR1, FLIPR2, FLIPR3) and emission filter for  $\text{Ca}^{2+}$  dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5  $\mu$ L below liquid level and dispense rate to 75  $\mu$ L/sec (96-well format) or 50  $\mu$ L/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 statistical significance.

## ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH3026802
HEPES 1M Stock	EMD Millipore.: TMS-003-C
Probenecid	Sigma: P8761
Quest Fluo-8 <sup>IM</sup> , AM	AAT Bioquest: 21080
U-50488 ligand	Sigma: D8040
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<sup>TETRA</sup>® 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 $\mu$ l (25 $\mu$ l for 384-well)
Dispense Height	25 $\mu$ l (50 $\mu$ l for 384-well)
Dispense Speed	75 $\mu$ l L/sec (50 $\mu$ l for 384-well)
Expel Volume	0 $\mu$ l
Analysis	Subtract Bias Sample 1

## HOST CELL

Chem-5, an adherent rat hematopoietic cell line expressing endogenous G $\cdot$ 15 protein as well as an exogenous proprietary promiscuous G $\alpha$  protein.

## EXOGENOUS GENE EXPRESSION

APJ cDNA (Accession Number: U03642; see CODING SEQUENCE below) expressed from a proprietary expressed from a proprietary PHS plasmid.

## CODING SEQUENCE

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1 - ATG GAG GAA GGT GGT GAT TTT GAC AAC TAC TAT GGG GCA GAC AAC CAG TCT GAG TGT GAG - 60
1 - M E E G G D F D N Y Y G A D N Q S E C E - 20

61 - TAC ACA GAC TGG AAA TCC TCG GGG GCC CTC ATC CCT GCC ATC TAC ATG TTG GTC TTC CTC - 120
21 - Y T D W K S S G A L I P A I Y M L V F L - 40

121 - CTG GGC ACC ACG GGA AAC GGT CTG GTG CTC TGG ACC GTG TTT CGG AGC AGC CGG GAG AAG - 180
41 - L G T T G N G L V L W T V F R S S R E K - 60

181 - AGG CGC TCA GCT GAT ATC TTC ATT GCT AGC CTG GCG GTG GCT GAC CTG ACC TTC GTG GTG - 240
61 - R R S A D I F I A S L A V A D L T F V V - 80

241 - ACG CTG CCC CTG TGG GCT ACC TAC ACG TAC CGG GAC TAT GAC TGG CCC TTT GGG ACC TTC - 300
81 - T L P L W A T Y T Y R D Y D W P F G T F - 100

301 - TTC TGC AAG CTC AGC AGC TAC CTC ATC TTC GTC AAC ATG TAC GCC AGC GTC TTC TGC CTC - 360
101 - L F T T S S Y L I F V N M Y A S V F C L - 120

361 - ACC GGC CTC AGC TTC GAC CGC TAC CTG GCC ATC GTG AGG CCA GTG GCC AAT GCT CGG CTG - 420
121 - T G L S F D R Y L A I V R P V A N A R L - 140

421 - AGG CTG CGG GTC AGC GGG GCC GTG GCC ACG GCA GTT CTT TGG GTG CTG GCC GCC CTC CTG - 480
141 - R L R V S G A V A T A V L W V L A A L L - 160

481 - GCC ATG CCT GTC ATG GTG TTA CGC ACC ACC GGG GAC TTG GAG AAC ACC ACT AAG GTG CAG - 540
161 - A M P V M V L R T T G D L E N T T K V Q - 180

541 - TGC TAC ATG GAC TAC TCC ATG GTG GCC ACT GTG AGC TCA GAG TGG GCC TGG GAG GTG GGC - 600
181 - C Y M D Y C S M V A T V S E W A W E V G - 200

601 - CTT GGG GTC TCG TCC ACC ACC GTG GGC TTT GTG GTG CCC TTC ACC ATC ATG CTG ACC TGT - 660
201 - L G V S S T T V G F V V P F T I M L T C - 220

661 - TAC TTC TTC ATC GCC CAA ACC ATC GCT GGC CAC TTC CGC AAG GAA CGC ATC GAG GGC CTG - 720
221 - Y F F I A Q T I A G H F R K E R I E G L - 240

721 - CGG AAG CGG CGC CGG CTG CTC AGC ATC ATC GTG GTG CTG GTG GTG ACC TTT GCC CTG TGC - 780
241 - R K R R R L L S I I V V L V V T F A L C - 260

781 - TGG ATG CCC TAC CAC CTG GTG AAG ACG CTG TAC ATG CTG GGC AGC CTG CTG CAC TGG CCC - 840
261 - W M P Y H L V K T L Y M L G S L L H W P - 280

841 - TGT GAC TTT GAC CTC TTC CTC ATG AAC ATC TTC CCC TAC TGC ACC TGC ATC AGC TAC GTC - 900
281 - C D F D L F L M N I F P Y C T C I S Y V - 300

901 - AAC AGC TGC CTC AAC CCC TTC CTC TAT GCC TTT TTC GAC CCC CGC TTC CGC CAG GCC TGC - 960
301 - N S C L N P F L Y A F F D P R F R Q A C - 320

961 - ACC TCC ATG CTC TGC TGT GGC CAG AGC AGG TGC GCA GGC ACC TCC CAC AGC AGC AGT GGG - 1020
321 - T S M L C C G Q S R C A G T S H S S S G - 340

1021 - GAG AAG TCA GCC AGC TAC TCT TCG GGG CAC AGC CAG GGG CCC GGC CCC AAC ATG GGC AAG - 1080
341 - E K S A S Y S S G H S Q G P G P N M G K - 360

1081 - GGT GGA GAA CAG ATG CAC GAG AAA TCC ATC CCC TAC AGC CAG GAG ACC CTT GTG GTT GAC - 1140
361 - G G E Q M H E K S I P Y S Q E T L V V D - 380

1141 - TGA
381 - *

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## RELATED PRODUCTS

### PRODUCT NUMBER

**HTSCHEM-1RTA**

**HTS068M**

### DESCRIPTION

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

ChemiScreen™ APJ Apelin receptor membrane prep

\* Note: Chem-5 cells are derived from Chem-1 cells

## REFERENCES

1. Habata Y et al. (1999) Apelin, the natural ligand of the orphan receptor APJ, is abundantly secreted in the colostrum. *Biochim. Biophys. Acta* 1452: 25-35.

2. Hosoya M et al. (2000) Molecular and functional characteristics of APJ: Tissue distribution of mRNA and interaction with the endogenous ligand apelin. *J. Biol. Chem.* 275: 21061–21067.
3. Katugampola SD et al. (2001) [125I]-(Pyr1)Apelin-13 is a novel radioligand for localizing the APJ orphan receptor in human and rat tissues with evidence for a vasoconstrictor role in man. *Br. J. Pharmacol.* 132: 1255-1260.
4. Lee DK et al. (2006) Unravelling the roles of the apelin system: prospective therapeutic applications in heart failure and obesity. *Trends Pharmacol. Sci.* 27: 190-194.
5. Medhurst AD et al. (2003) Pharmacological and immunohistochemical characterization of the APJ receptor and its endogenous ligand apelin. *J. Neurochem.* 84: 1162-1172.
6. Reaux A et al. (2001) Physiological role of a novel neuropeptide, apelin, and its receptor in the rat brain. *J. Neurochem.* 77: 1085-1096.

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