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PRODUCT DATASHEET

Ready-to-Assay™ TSH Glycoprotein Hormone Receptor Frozen Cells

CATALOG NUMBER: HTS133RTA

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_2. Media Component at 4ºC (-20ºC 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.

The glycoprotein hormone receptor family consists of the luteinizing hormone receptor, the follicle-stimulating hormone receptor, and the thyroid stimulating hormone (TSH) receptor. TSH, which is released from the pituitary gland, binds to the TSH receptor on thyroid cells to control size and function of the thyroid gland (De Felice et al., 2004). The TSH receptor signals through Gs to elevate intracellular cAMP in the thyroid gland, which regulates iodide uptake, and transcription of thyroglobulin (Tg), thyroid peroxidase (TPO), and sodium-iodide symporter. The TSH receptor also signals Gq and phospholipase C to regulate iodide efflux, H_2O_2 production, and thyroglobulin iodination. Autoimmunity to the TSH receptor causes hyperthyroidism (Graves disease) or hypothyroidism (Hashimoto thyroiditis) when the autoantibodies function as agonists or antagonists, respectively, at the TSH receptor (Rapoport and McLachlan, 2001; Davies et al., 2002). Cloned human TSH-expressing cell line is made in the Chem-10 host, which supports high levels of recombinant TSH 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 TSH.

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.

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

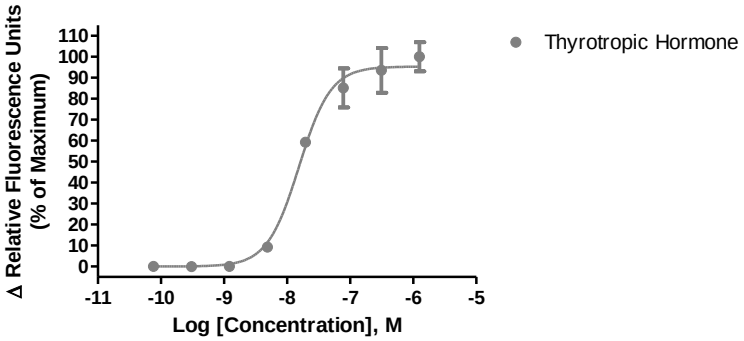

Figure 1. Representative data for activation of TSH receptor. Calcium flux in TSH–expressing Chem-10 cell line induced by Thyrotropic Hormone. TSH–expressing Chem-10 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices FLIPR^{TETRA} with ICCD camera. Maximal fluorescence signal obtained in this experiment was 22,500 RLU (Relative Light Units).

Table 1. EC_{50} value of TSH-expressing Chem-10 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
Thyrotropic Hormone	Calcium Flux	16	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 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. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 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) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} 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 Ca^{2+} 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
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
Thyrotropic Hormone ligand	Sigma: T8931
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-10, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein as well as an exogenous proprietary promiscuous Gα protein.

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EXONGENOUS GENE EXPRESSION

Human TSHR cDNA (Accession Number: NM_000369; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

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   1 - ATGAGGCCGGCGGACTTGCTGCAGCTGGTGCTGCTGCTCGACCTGCCCAGGGACCTGGGCGGAATGGGGTGTTCGTCTCCACCCTGCGAG - 90
   1 -  M  R  P  A  D  L  L  Q  L  V  L  L  L  D  L  P  R  D  L  G  G  M  G  C  S  S  P  P  C  E  - 30

  91 - TGCCATCAGGAGGAGGACTTCAGAGTCACCTGCAAGGATATTCAACGCATCCCCAGCTTACCGCCCAGTACGCAGACTCTGAAGCTTATT - 180
  31 -  C  H  Q  E  E  D  F  R  V  T  C  K  D  I  Q  R  I  P  S  L  P  P  S  T  Q  T  L  K  L  I  - 60

 181 - GAGACTCACCTGAGAACTATTCCAAGTCATGCATTTTCTAATCTGCCCAATATTTCCAGAATCTACGTATCTATAGATGTGACTCTGCAG - 270
  61 -  E  T  H  L  R  T  I  P  S  H  A  F  S  N  L  P  N  I  S  R  I  Y  V  S  I  D  V  T  L  Q  - 90

 271 - CAGCTGGAATCACACTCCTTCTACAATTTGAGTAAAGTGACTCACATAGAAATTCGGAATACCAGGAACTTAACTTACATAGACCCTGAT - 360
  91 -  Q  L  E  S  H  S  F  Y  N  L  S  K  V  T  H  I  E  I  R  N  T  R  N  L  T  Y  I  D  P  D  - 120

 361 - GCCCTCAAAGAGCTCCCCCTCCTAAAGTTCCTTGGCATTTTCAACACTGGACTTAAAATGTTCCCTGACCTGACCAAAGTTTATTCCACT - 450
 121 -  A  L  K  E  L  P  L  L  K  F  L  G  I  F  N  T  G  L  K  M  F  P  D  L  T  K  V  Y  S  T  - 150

 451 - GATATATTCTTTATACTTGAAATTACAGACAACCCTTACATGACGTCAATCCCTGTGAATGCTTTTCAGGGACTATGCAATGAAACCTTG - 540
 151 -  D  I  F  F  I  L  E  I  T  D  N  P  Y  M  T  S  I  P  V  N  A  F  Q  G  L  C  N  E  T  L  - 180

 541 - ACACTGAAGCTGTACAACAATGGCTTTACTTCAGTCCAAGGATATGCTTTCAATGGGACAAAGCTGGATGCTGTTTACCTAAACAAGAAT - 630
 181 -  T  L  K  L  Y  N  N  G  F  T  S  V  Q  G  Y  A  F  N  G  T  K  L  D  A  V  Y  L  N  K  N  - 210

 631 - AAATACCTGACAGTTATTGACAAAGATGCATTTGGAGGAGTATACAGTGGACCAAGCTTGCTGGACGTGTCTCAAACCAGTGTCACTGCC - 720
 211 -  K  Y  L  T  V  I  D  K  D  A  F  G  G  V  Y  S  G  P  S  L  L  D  V  S  Q  T  S  V  T  A  - 240

 721 - CTTCCATCCAAAGGCCTGGAGCACCTGAAGGAACTGATAGCAAGAAACACCTGGACTCTTAAGAAACTTCCACTTTCCTTGAGTTTCCTT - 810
 241 -  L  P  S  K  G  L  E  H  L  K  E  L  I  A  R  N  T  W  T  L  K  K  L  P  L  S  L  S  F  L  - 270

 811 - CACCTCACACGGGCTGACCTTTCTTACCCAAGCCACTGCTGTGCTTTTAAGAATCAGAAGAAAATCAGAGGAATCCTTGAGTCCTTGATG - 900
 271 -  H  L  T  R  A  D  L  S  Y  P  S  H  C  C  A  F  K  N  Q  K  K  I  R  G  I  L  E  S  L  M  - 300

 901 - TGTAATGAGAGCAGTATGCAGAGCTTGCGCCAGAGAAAATCTGTGAATGCCTTGAATAGCCCCCTCCACCAGGAATATGAAGAGAATCTG - 990
 301 -  C  N  E  S  S  M  Q  S  L  R  Q  R  K  S  V  N  A  L  N  S  P  L  H  Q  E  Y  E  E  N  L  - 330

 991 - GGTGACAGCATTGTTGGGTACAAGGAAAAGTCCAAGTTCCAGGATACTCATAACAACGCTCATTATTACGTCTTCTTTGAAGAACAAGAG - 1080
 331 -  G  D  S  I  V  G  Y  K  E  K  S  K  F  Q  D  T  H  N  N  A  H  Y  Y  V  F  F  E  E  Q  E  - 360

1081 - GATGAGATCATTGGTTTTGGCCAGGAGCTCAAAAACCCCCAGGAAGAGACTCTACAAGCTTTTGACAGCCATTATGACTACACCATATGT - 1170
 361 -  D  E  I  I  G  F  G  Q  E  L  K  N  P  Q  E  E  T  L  Q  A  F  D  S  H  Y  D  Y  T  I  C  - 390

1171 - GGGGACAGTGAAGACATGGTGTGTACCCCCAAGTCCGATGAGTTCAACCCGTGTGAAGACATAATGGGCTACAAGTTCCTGAGAATTGTG - 1260
 391 -  G  D  S  E  D  M  V  C  T  P  K  S  D  E  F  N  P  C  E  D  I  M  G  Y  K  F  L  R  I  V  - 420

1261 - GTGTGGTTCGTTAGTCTGCTGGCTCTCCTGGGCAATGTCTTTGTCCTGCTTATTCTCCTCACCAGCCACTACAAACTGAACGTCCCCCGC - 1350
 421 -  V  W  F  V  S  L  L  A  L  L  G  N  V  F  V  L  L  I  L  L  T  S  H  Y  K  L  N  V  P  R  - 450

1351 - TTTCTCATGTGCAACCTGGCCTTTGCGGATTTCTGCATGGGGATGTACCTGCTCCTCATCGCCTCTGTAGACCTCTACACTCACTCTGAG - 1440
 451 -  F  L  M  C  N  L  A  F  A  D  F  C  M  G  M  Y  L  L  L  I  A  S  V  D  L  Y  T  H  S  E  - 480

1441 - TACTACAACCATGCCATCGACTGGCAGACAGGCCCTGGGTGCAACACGGCTGGTTTCTTCACTGTCTTTGCAAGCGAGTTATCGGTGTAT - 1530
 481 -  Y  Y  N  H  A  I  D  W  Q  T  G  P  G  C  N  T  A  G  F  F  T  V  F  A  S  E  L  S  V  Y  - 510

1531 - ACGCTGACGGTCATCACCCTGGAGCGCTGGTATGCCATCACCTTCGCCATGCGCCTGGACCGGAAGATCCGCCTCAGGCACGCATGTGCC - 1620
 511 -  T  L  T  V  I  T  L  E  R  W  Y  A  I  T  F  A  M  R  L  D  R  K  I  R  L  R  H  A  C  A  - 540

1621 - ATCATGGTTGGGGGCTGGGTTTGCTGCTTCCTTCTCGCCCTGCTTCCTTTGGTGGGAATAAGTAGCTATGCCAAAGTCAGTATCTGCCTG - 1710
 541 -  I  M  V  G  G  W  V  C  C  F  L  L  A  L  L  P  L  V  G  I  S  S  Y  A  K  V  S  I  C  L  - 570

1711 - CCCATGGACACCGAGACCCCTCTTGCTCTGGCATATATTGTTTTTGTTCTGACGCTCAACATAGTTGCCTTCGTCATCGTCTGCTGCTGT - 1800
 571 -  P  M  D  T  E  T  P  L  A  L  A  Y  I  V  F  V  L  T  L  N  I  V  A  F  V  I  V  C  C  C  - 600

1801 - TATGTGAAGATCTACATCACAGTCCGAAATCCGCAGTACAACCCAGGGGACAAAGATACCAAAATTGCCAAGAGGATGGCTGTGTTGATC - 1890
 601 -  Y  V  K  I  Y  I  T  V  R  N  P  Q  Y  N  P  G  D  K  D  T  K  I  A  K  R  M  A  V  L  I  - 630
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1891 - TTCACCGACTTCATATGCATGGCCCCAATCTCATTCTATGCTCTGTCAGCAATTCTGAACAAGCCTCTCATCACTGTTAGCAACTCCAAA - 1980
 631 -  F  T  D  F  I  C  M  A  P  I  S  F  Y  A  L  S  A  I  L  N  K  P  L  I  T  V  S  N  S  K  - 660

1981 - ATCTTGCTGGTACTCTTCTATCCACTTAACTCCTGTGCCAATCCATTCCTCTATGCTATTTTCACCAAGGCCTTCCAGAGGGATGTGTTC - 2070
 661 -  I  L  L  V  L  F  Y  P  L  N  S  C  A  N  P  F  L  Y  A  I  F  T  K  A  F  Q  R  D  V  F  - 690

2071 - ATCCTACTCAGCAAGTTTGGCATCTGTAAACGCCAGGCTCAGGCATACCGGGGGGCAGAGGGTTCCTCCAAAGAACAGCACTGATATTCAG - 2160
 691 -  I  L  L  S  K  F  G  I  C  K  R  Q  A  Q  A  Y  R  G  Q  R  V  P  P  K  N  S  T  D  I  Q  - 720

2161 - GTTCAAAAGGTTACCCACGACATGAGGCAGGGTCTCCACAACATGGAAGATGTCTATGAACTGATTGAAAACTCCCATCTAACCCCAAAG - 2250
 721 -  V  Q  K  V  T  H  D  M  R  Q  G  L  H  N  M  E  D  V  Y  E  L  I  E  N  S  H  L  T  P  K  - 750

2251 - AAGCAAGGCCAAATCTCAGAAGAGTATATGCAAACGGTTTTG TAA - 2292
 751 -  K  Q  G  Q  I  S  E  E  Y  M  Q  T  V  L  Stp - 765
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RELATED PRODUCTS

PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)

Note: Chem-10 cells are derived from Chem-1 cells.

REFERENCES

1. Davies et al,. (2004) Thyrotropin Receptor Signaling in development and Differentiation of the Thyroid Gland: Insights from Mouse Models and Human Diseases. *Endocronoligy*.145(9):4062-4067.
2. De Felice et al,. (2004) The TSH receptor reveals itself. *The J. Clin Inves*.110:161-164.
3. Rapoport et al,. (2001) Thyroid autoimmunity. The *J. Clin Inves*.108:1253-1259.

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