Human Parathyroid Hormone Receptor 2 Stable Cell Line

Technical Manual No. TM0298 **Version 05132009**

I	Introduction	1
II	Cell Line Information.....	1
III	PTH (1-34) Dose Response of HEK293/PTHR2/CRE/β-Lac Stable Cell Line and Assay Procedure	2
IV	References	3
V	Appendix	3
	User Agreement (Label License).....	9

I. Introduction

The parathyroid hormone receptor 2 (PTHR2) is a member of G-protein coupled receptor family 2. This protein is a receptor for parathyroid hormone (PTH). This receptor is more selective in ligand recognition and has a more specific tissue distribution than parathyroid hormone receptor 1 (PTHR1). It is activated only by PTH and not by parathyroid hormone-like hormone (PTHLH) and is particularly abundant in the brain and pancreas.

This manual describes establishment of a cell line and a protocol of pharmacologically validated human PTHR2 GPCR receptor (Genebank Accession Number: NM_005048).

II. Cell Line Information

- Catalog Number: M00141
- Cell Line Name: HEK293/PTHR2/CRE/β-Lac
- Description:
  The HUMAN PTHR2 is amplified by PCR using a high fidelity enzyme and subcloned into the pcDNA3.1-hygro (+) mammalian expression vector. The full-length ORF has been confirmed by sequencing. The PTHR2 reporter cell line is created by cotransfection of pcDNA3.1-hygro(+)/PTHR2 and pCRE/β-Lac in an HEK293 cell line. The transfected cells are stably selected by 100 μg/ml hygromycin. Single cell clones with high PTHR2 inducibility and low β-lactamase background are isolated using ring cloning. The clones with the largest dynamic ranges in β-lactamase are chosen for pharmacological and stability studies.
- Function: Cell-based, functional assay for PTHR2
- Quantity: 2 vial (2 x 10^6) frozen cells
- Host Cell: HEK293
- Cell Phenotype: Adherent/epithelial
- Recommended Storage: Liquid nitrogen, upon delivery
- Propagation Medium: DMEM, 10% FBS, 100 μg/ml hygromycin
- Mycoplasma: Negative

120 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com

III. PTH (1-34) Dose Response of HEK293/PTHR2/CRE/β-Lac Stable Cell Line and Assay Procedure

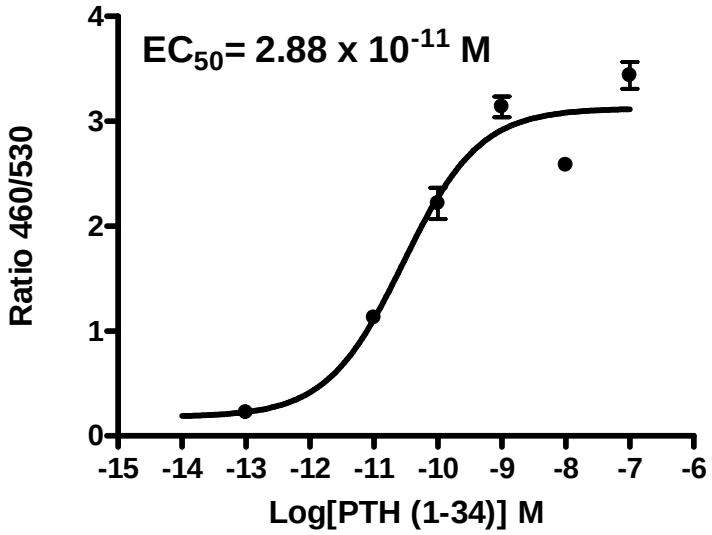


Fig. 1. Dose response of β-lactamase activity as monitored with Analyst HT plate reader upon treatment with ligand. Assay was done according to procedure described as below. Data represent means ± SEM for triplicate samples. EC_{50} value for PTH (1-34) dose response was determined using GraphPad Prism 4 software.

Assay Procedure

CCF-4 Assay of HEK293/PTHR2/CRE/β-Lac Reporter Cells

1. Seed 25,000 cells per well in Growth Medium (100 μL per well) into 96-well tissue culture treated black-wall, clear-bottom plates (Costar #3603) after trypsinization. Prepare some wells with medium alone (no cells) to use for determining plate background.
2. Culture cells in 5% CO_2 at 37°C. Allow cells to reach ≈90% confluence.
3. 12-24 hours before the assay, replace Growth Medium with 100 μL/well serum-free DMEM. Be careful not to disturb the cells.
4. Prepare ligand solution in serum-free DMEM (10X).
5. Add 10 μL of 10X PTH (1-34) solution to wells for stimulation and 10 μL of serum-free DMEM per well for non-stimulated control.
6. Incubate cells in 5% CO_2 at 37°C for five to six hours.
7. Load cells with 2 μM CCF4/AM as described in CCF4 Loading Protocol.
8. Incubate the plate at room temperature for 60-120 minutes without shaking.
9. Wait 60-200 minutes after CCF-4 loading. Then either read with Analyst HT plate reader or observe under fluorescence microscope.

CCF-4 Loading Protocol

1. Solution A: Dissolve 5 mg CCF-4/AM with 4.6 ml DMSO to a stock concentration at 1 mM. Aliquot and store at -80°C, protected from light.
2. Solution B: 100 mg/ml Pluronic -F127 surfactant in DMSO and 0.1% acetic acid
3. Solution C: Red Dye solution
4. 6X substrate loading solution:

120 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com

1) Add 6 µL of Solution A to 60 µL of Solution B and mix well.
2) Add 934 µL of Solution C to the combined Solutions A and B and mix well.

5. Add 6X Substrate Loading Solution to cells to 1X final concentration (e.g., add 20 µL of 6XCCF4-AM Substrate Loading Solution to 100 µL of cells in buffer).
6. Add the same volume of 6X Substrate Loading Solution to the cell-free background control wells containing assay medium or buffer to 1X final concentration.

Cover the plate to protect it from light and evaporation.

IV. References

1. ALESSANDRO BISELLO *et al*. (2004) Agonist-Specific Regulation of Parathyroid Hormone (PTH) Receptor Type 2 Activity: Structural and Functional Analysis of PTH- and Tuberoinfundibular Peptide (TIP) 39-Stimulated Desensitization and Internalization.2004. *Molecular Endocrinology* 18: 1486–1498.

2. Thomas J. Gardella and Harald Jüppner. (2001) Molecular properties of the PTH/PTHrP receptor. July 2001, *TRENDS in Endocrinology & Metabolism* Vol.12 No.5

V. Appendix

Cell Culture Conditions

Note: This clone often grows as clumps. It is necessary to separate cells into single cell suspensions whenever the cells are passaged or plated. MATRIGEL Matrix-coated flasks or plates are recommended for healthier cells.

Complete Culture Medium:

DMEM: 90%, FBS: 10%, L-glutamine: 2.0 mM, Amp: 100 µg/ml, Strep:100 µg/ml, hygromycin: 50 µg/ml.

Freezing Medium:

Growth medium plus 20% FBS and 10% DMSO

Thawing Cells:

1. Quickly thaw frozen cells in a 37°C water bath, agitating continuously.
2. Using a 1 ml pipette, slowly pipet the cells up and down five times and add, drop by drop, to a 15 ml centrifuge tube containing 5 ml of fresh prewarmed complete DMEM medium. Then centrifuge at 1,000 rpm for five minutes.
3. Discard the supernatant medium and resuspend the cell pellet in 5 ml of fresh prewarmed complete DMEM medium. Transfer cells to a T25 flask and incubate at 37°C with 5% CO_2 until the cells reach >90% confluence. The recovery rate for frozen cells is usually 90% or above.

Subculturing:

When the cells reach confluence, they need split. This cell line is normally split twice weekly at 1:8 to 1:15 dilutions.

1. Carefully aspirate all the media. Gently rinse the cell layer with appropriate amount of 0.2% trypsin-EDTA, and aspirate it off.
2. Wait for about 1-3 minutes. Then dislodge the cells by gently tapping the sides of flask or dish.
3. Resuspend cells with appropriate amount of complete DMEM medium, and split cells as desired.

120 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com

Changing Medium:

This is normally done every other day.

1. Gently aspirate off medium.
2. Transfer fresh warm complete DMEM medium (37°C) into a flask (5 ml for T25 and 10 ml for T75).

Freezing Cells:

1. Repeat the steps 1-3 of subculturing section.
2. Centrifuge down the cells at 1,000 rpm for five minutes.
3. Aspirate off the supernatant and resuspend the cells in fresh freezing medium at a density of 2-3 × 10^6 cells/ml. Add 1 ml cells per cryogenic vial.
4. Put the cryogenic vial of cells into cryo freezing container. Then transfer the container to a -80°C environment and leave it there overnight.
5. Transfer cryogenic vial into liquid nitrogen (-196°C).

Reagents & Consumables:

1. DMEM: Dulbecco's Modified Eagle Medium powder, high glucose (Gibco BRL, Cat #12100-046)
2. FBS: Fetal Bovine Serum (Hyclone, Cat #CH30160.03)
3. L-Glutamine: 200 mM (Gibco BRL, Cat # 25030-081)
4. Ampicillin: 50 mg/ml (Sigma A-9518)
5. Streptomycin Sulfate: 50 mg/ml (Gibco BRL, Cat # 11860-038)
6. Hygromycin B in PBS, 50 mg/ml (Invitrogen, Cat #10687-010)
7. Trypsin: 1:250 rom Bovine Pancreas (Gibco BRL, Cat # 27250016)
8. DMSO: dimethyl sulphoxide, for molecular biology (Sigma, Cat #D8418)
9. Hepes: Sigma Cat #H-3375
10. CCF4: (Invitrogen, Cat #K1096)
11. PTH (1-34): synthesized by HD Biosciences
12. Cryogenic Vial: (Corning Cat #430289)
13. 96 Well Plate: Costar, Cat# 3603, Blackwall/clear bottom, Polystyrene, sterilized.

Media and Solutions:

1. PBS (for preparation of 500 ml)

1)	KCl:	0.1 g
2)	KH_2PO_4:	0.1 g
3)	NaCl:	4.0 g
4)	$Na_2HPO_4.12H_2O$:	1.4425 g

Dissolve the above components in double-distilled water (ddH_2O) and adjust pH to 7.4 with 0.1 N NaOH. Add ddH_2O to the final volume of 500 ml. Autoclave and store at 4°C.

120 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com

2. Trypsin-EDTA (for preparation of 100 ml)

1)	Trypsin:	0.25 g
2)	2%EDTA:	2 ml
3)	PBS:	98 ml

Dissolve trypsin in 2%EDTA and PBS completely. Sterilize the solution by passing through a 0.20 μm membrane filter; store at 4°C.

3. Culture medium (for preparation of 1 L)

1) Measure out 950 ml distilled water to dissolve the media components, stirring gently until the solution becomes clear.
2) Add $NaHCO_3$ 3.7 g for high glucose DMEM.
3) Adjust pH of medium to 0.2-0.3 below the desired final working pH (using 1 N NaOH or 1 N HCL is recommended). Add slowly while stirring.
4) Dilute to 1 liter with ddH_2O.
5) Sterilize the medium immediately using the method of membrane filtration. Store at 4°C.

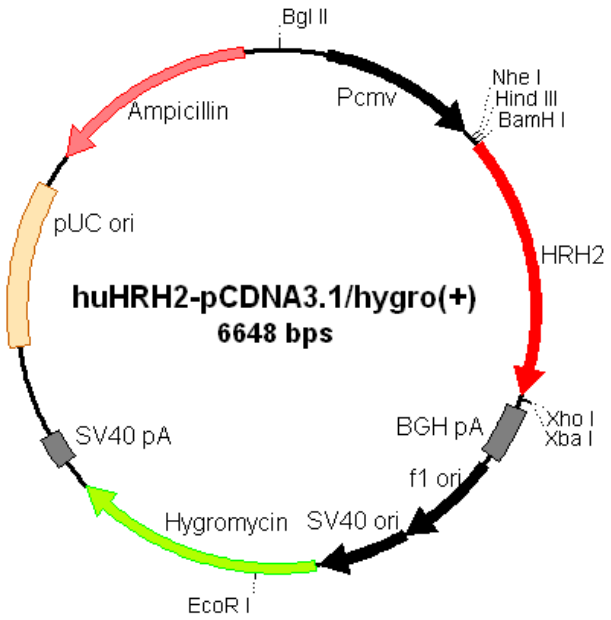
4. Ampicillin/streptomycin 50 mg/ml

Dissolve 1 g ampicillin or streptomycin in 20 ml ddH_2O and sterilize the solution by membrane filtration using 0.20 μm filter. Aliquot and store at 4°C for short-term conservation and -20°C for long term conservation.

Map of huHRH2-pCDNA3.1/hygro(+)

Name: huHRH2-pCDNA3.1/hygro(+)
Insert gene: *Homo sapiens* histamine receptor H2, (HRH2)
Length of insert: 1103bp Sequence reference: Gene Bank NM_022304
Vector: pCDNA3.1/hygro(+) (Invitrogen) Insert site: BamH I (5') and XhoI (3')

Plasmid Map:



120 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com

Comments for huHRH2-pCDNA3.1/hygro(+) 6648nucleotides

CMV promoter: bases 232~819
T7 promoter/priming site: bases 863~882
huHRH2 gene: bases 938~2020
pCDNA3.1/BGH reverse priming site: bases 2069~2086
BGH polyadenylation signal: bases 2075~2299
f1 origin: bases 2345~2773
SV40 early promoter and origin: bases 2778~3148
Hygromycin resistance gene (ORF): bases 3166~4191
SV40 early polyadenylation signal: bases 4321~4451
pUC origin: bases 4834~5507 (complementary strand)
Ampicillin resistance gene (bla): bases 5652~6512 (complementary strand)

Name: Human histamine receptor H2 (HRH2) in pCDNA3.1/hygro(+)

GACGGATCGGGAGATCTCCCGATCCCCTATGGTCGACTCTCAGTACAATCTGCTCTGATGCCGCATAGTTAAG
CCAGTATCTGCTCCCTGCTTGTGTGTTGGAGGTCGCTGAGTAGTGCGCGAGCAAAATTTAAGCTACAACAAGG
CAAGGCTTGACCGACAATTGCATGAAGAATCTGCTTAGGGTTAGGCGTTTTGCGCTGCTTCGCGATGTACGG
GCCAGATATACGCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGC
CCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGC
CCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGG
ACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTC
AATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACA
TCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTT
TGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGG
GACTTTCCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTC
TATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAATACGACTCACTA
TAGGGAGACCCAAGCTGGCTAGCGTTTAAACTTAAGCTTGGTACCGAGCTCGGATCCACCATGGCACCCAAT
GGCACAGCCTCTTCCTTTTGCCTGGACTCTACCGCATGCAAGATCACCATCACCGTGGTCCTTGCGGTCCTCA
TCCTCATCACCGTTGCTGGCAATGTGGTCGTCTGTCTGGCCGTGGGCTTGAACCGCCGGCTCCGCAACCTGA
CCAATTGTTTCATCGTGTCCTTGGCTATCACTGACCTGCTCCTCGGCCTCCTGGTGCTGCCCTTCTCTGCCAT
CTACCAGCTGTCCTGCAAGTGGAGCTTTGGCAAGGTCTTCTGCAATATCTACACCAGCCTGGATGTGATGCTC
TGCACAGCCTCCATTCTTAACCTCTTCATGATCAGCCTCGACCGGTACTGCGCTGTCATGGACCCACTGCGGT
ACCCTGTGCTGGTCACCCCAGTTCGGGTCGCCATCTCTCTGGTCTTAATTTGGGTCATCTCCATTACCCTGTC
CTTTCTGTCTATCCACCTGGGGTGGAACAGCAGGAACGAGACCAGCAAGGGCAATCATACCACCTCTAAGTG
CAAAGTCCAGGTCAATGAAGTGTACGGGCTGGTGGATGGGCTGGTCACCTTCTACCTCCCGCTACTGATCAT
GTGCATCACCTACTACCGCATCTTCAAGGTCGCCCGGGATCAGGCCAAGAGGATCAATCACATTAGCTCCTG
GAAGGCAGCCACCATCAGGGAGCACAAAGCCACAGTGACACTGGCCGCCGTCATGGGGGCCTTCATCATCT
GCTGGTTTCCCTACTTCACCGCGTTTGTGTACCGTGGGCTGAGAGGGGATGATGCCATCAATGAGGTGTTAG
AAGCCATCGTTCTGTGGCTGGGCTATGCCAACTCAGCCCTGAACCCCATCCTGTATGCTGCGCTGAACAGAG
ACTTCCGCACCGGGTACCAACAGCTCTTCTGCTGCAGGCTGGCCAACCGCAACTCCCACAAAACTTCTCTGA

120 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com

GGTCCAACGCCTCTCAGCTGTCCAGGACCCAAAGCCGAGAACCCAGGCAACAGGAAGAGAAACCCCTGAAG
CTCCAGGTGTGGAGTGGGACAGAAGTCACGGCCCCCCAGGGAGCCACAGACAGGTAATAGCCCTAGCCATT
CTCGAGTCTAGAGGGCCCGTTTAAACCCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTG
TTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGA
AATTGCATCGCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGA
GGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGCTTCTGAGGCGGAAAGAACCA
GCTGGGGCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTAC
GCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCC
ACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGCATCCCTTTAGGGTTCCGATTTAGTGCTTTACGGC
ACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCG
CCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCT
CGGTCTATTCTTTTGATTTATAAGGGATTTTGGGGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAA
AATTTAACGCGAATTAATTCTGTGGAATGTGTGTCAGTTAGGGTGTGGAAAGTCCCCAGGCTCCCCAGGCAGG
CAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCAGGTGTGGAAAGTCCCCAGGCTCCCCAGCAGG
CAGAAGTATGCAAAGCATGCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCCATCCCGCCC
CTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGG
CCGCCTCTGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCT
CCCGGGAGCTTGTATATCCATTTTCGGATCTGATCAGCACGTGATGAAAAAGCCTGAACTCACCGCGACGTCT
GTCGAGAAGTTTCTGATCGAAAAGTTCGACAGCGTCTCCGACCTGATGCAGCTCTCGGAGGGCGAAGAATCT
CGTGCTTTCAGCTTCGATGTAGGAGGGCGTGGATATGTCCTGCGGGTAAATAGCTGCGCCGATGGTTTCTAC
AAAGATCGTTATGTTTATCGGCACTTTGCATCGGCCGCGCTCCCGATTCCGGAAGTGCTTGACATTGGGGAAT
TCAGCGAGAGCCTGACCTATTGCATCTCCCGCCGTGCACAGGGTGTCACGTTGCAAGACCTGCCTGAAACCG
AACTGCCCGCTGTTCTGCAGCCGGTCGCGGAGGCCATGGATGCGATCGCTGCGGCCGATCTTAGCCAGACG
AGCGGGTTCGGCCCATTCGGACCGCAAGGAATCGGTCAATACACTACATGGCGTGATTTCATATGCGCGATT
GCTGATCCCCATGTGTATCACTGGCAAACTGTGATGGACGACACCGTCAGTGCGTCCGTCGCGCAGGCTCTC
GATGAGCTGATGCTTTGGGCCGAGGACTGCCCCGAAGTCCGGCACCTCGTGCACGCGGATTTCGGCTCCAA
CAATGTCCTGACGGACAATGGCCGCATAACAGCGGTCATTGACTGGAGCGAGGCGATGTTCGGGGATTCCCA
ATACGAGGTCGCCAACATCTTCTTCTGGAGGCCGTGGTTGGCTTGTATGGAGCAGCAGACGCGCTACTTCGA
GCGGAGGCATCCGGAGCTTGCAGGATCGCCGCGGCTCCGGGCGTATATGCTCCGCATTGGTCTTGACCAAC
TCTATCAGAGCTTGGTTGACGGCAATTTCGATGATGCAGCTTGGGCGCAGGGTCGATGCGACGCAATCGTCC
GATCCGGAGCCGGGACTGTCGGGCGTACACAAATCGCCCGCAGAAGCGCGGCCGTCTGGACCGATGGCTGT
GTAGAAGTACTCGCCGATAGTGGAAACCGACGCCCCAGCACTCGTCCGAGGGCAAAGGAATAGCACGTGCTA
CGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTTGGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGG
ATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCCACCCCAACTTGTTTATTGCAGCTTATAATG
GTTACAAATAAAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGT
CCAAACTCATCAATGTATCTTATCATGTCTGTATACCGTCGACCTCTAGCTAGAGCTTGGCGTAATCATGGTCA
TAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAA

120 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com

AGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGG
AAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCT
CTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAA
AGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAA
AGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACA
AAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAA
GCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAA
GCGTGGCGCTTTCTCAATGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCT
GTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGG
TAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTG
CTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCT
GAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGG
TTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGG
GGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCAC
CTAGATCCTTTTAAATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTAAACTTGGTCTGACAGTTAC
CAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGT
CGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACG
CTCACCGGCTCCAGATTTATCAGCAATAAACCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAAC
TTTATCCGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGC
GCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGG
TTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCTCCG
ATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGT
CATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGG
CGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCA
TCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACC
CACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGG
CAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTA
TTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGG
GGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGT

GenScript USA Inc.
120 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: info@genscript.com
Web: http://www.genscript.com

For Research Use Only.

User Agreement (Label License) for Human Parathyroid Hormone Receptor 2 Cell Line

Catalog Number: M00141

This is a legal agreement between you (Licensee) and GenScript USA Inc. governing use of GenScript's Stable GPCR cell line products and protocols provided to licensee. By using this product licensed to the licensee, the licensee is consenting to be legally bound by all of the terms of this user agreement. The licensee will obtain no ownership interest in the product or any use/culture protocols accompanying the product other than through the perpetual limited license granted herein. If the product is licensed through an authorized GenScript distributor, the licensee will be obligated to disclose its identity to GenScript to ensure compliance with this user agreement.

All products sold by GenScript are for laboratory and animal research purposes only. The products are not to be used on humans, for consumption, or for any unlawful uses.

The licensee agrees, (i) that it shall use all products solely in accordance with this agreement, (ii) that any such use of products will not violate any applicable laws, regulations, judicial orders, or injunctions; and (iii) that it is not prohibited from receiving the products under U.S. export laws, that it is not a national of a country subject to U.S. trade sanctions, that it will not use the products in a location that is the subject of U.S. trade sanctions that would cover the products, and that, to its knowledge, it is not on the U.S. Department of Commerce's table of deny orders or is otherwise prohibited from obtaining goods of this sort from the United States.

GenScript USA Inc. will not assert against the buyer a claim of infringement of patents owned or controlled by GenScript USA Inc. and claiming this product based upon the manufacture, use or sale of a clinical diagnostic, therapeutic and vaccine, or prophylactic product developed in research by the buyer in which this product or its components has been employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on the use of this product for other purposes, contact Marketing Department, GenScript USA Inc., 120 Centennial Avenue, Piscataway, New Jersey 08840, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: marketing@genscript.com.

120 Centennial Ave., Piscataway, NJ 08854, USA
Tel: 1-732-885-9188 Fax: 1-732-210-0262 Email: info@genscript.com Web: www.genscript.com