

Technical Data Sheet

Codex Human Thyroid Stimulating Hormone Receptor (TSHR) Cell Line**Product Information**

Catalog Number: **CB-80200-242b**
Components: **TSHR cell line, 1 vials, frozen**

Description

cAMP is a key second messenger involved extensively in cellular signal transduction pathways associated with the majority of G-protein coupled receptors (GPCRs). The activation of these GPCRs by neurotransmitters, lipids, nucleotides, peptides and hormones results in the activation or the inhibition of plasma membrane-bound adenylate cyclase through heterotrimeric G-proteins.

TSHR is the receptor for thyrotropin (thyroid stimulating hormone or TSH), a member of the glycoprotein hormone family. TSH is released by the anterior pituitary gland and is the main regulator of thyroid gland growth and development. Upon binding circulating thyrotropin, a G-protein signal cascade activates adenyl cyclase and intercellular levels of cAMP rise. cAMP activates all functional aspects of the thyroid cell, including iodine pumping; thyroglobulin synthesis, iodination, endocytosis and proteolysis; thyroid peroxidase activity; and hormone release.

Storage

Frozen TSHR cells should be stored in liquid nitrogen.

Assay materials not included

ACTOne™ cAMP Fluorimetric ELISA Assay Kit (384-well plate)
Biocoat Poly-D-Lysine coated 384-well black/clear plate
Phosphodiesterase (PDE) inhibitor Ro 20-1724 (50mM stock in DMSO, store at -20°C)
Dulbecco's Phosphate Buffered Saline (DPBS)
TSH (1 µg/µl stock in dH₂O)

Codex CB-80500-523
BD 354663
Sigma B8279
Sigma D8537
Fitzgerald Industries International, Inc,
30-AT09

Cell culture materials not included

DMEM, high glucose, with glutamine
Fetal bovine serum
Trypsin-EDTA solution (10x)
Puromycin

Invitrogen 11995-065
Invitrogen 26140-079
Sigma T4174
Invitrogen A11138-03

CELL CULTURE PROTOCOL

Note. Please finish reading the whole protocol before beginning the experiment.

THAWING AND PLATING CELLS (REQUIRES 1-3 DAYS)

1. Prepare complete cell culture medium consisting of 90% Dulbecco's Modified Eagle Medium (DMEM), 10% fetal bovine serum (FBS) and 1 µg/ml puromycin. Warm the medium to ~37°C.
2. Remove a vial of cells from the liquid Nitrogen tank. Wear safety glasses and always point the cap away from your face when opening.
3. Place the vial of cells in a 37°C water bath until just thawed (less than 5 minutes). Immediately transfer cells to a 10 cm cell-culture plate or a T25 flask with 9 ml of the appropriate culture medium (pre-warmed to 37°C).
4. Place the cells in a cell-culture incubator at 37°C with 5% CO₂ for 4 hrs.
5. After 4 hours replace the culture medium with appropriate fresh culture medium (pre-warmed at 37°C).
6. Place the cells back in the incubator for 1-3 days. The cells will not require feeding before they reach 80-90% confluence and are ready for expansion. Split the cells when they reach 80-90% confluence.

Note. It is very important that the cells DO NOT reach >90% confluence. Over-confluent growth can result in a significantly reduced response to ligands, and it may take several passages for the cells to recover to optimal stage.

SPLITTING AND AMPLIFYING CELLS (REQUIRES 1-3 DAYS)

1. Remove the culture medium and replace it with a volume of Dulbecco's Phosphate Buffers Saline without calcium and magnesium (DPBS) to adequately cover and wash the cells. Remove DPBS.
2. Add a sufficient volume of 1x trypsin-EDTA to just cover the cells (i.e. 1 ml for a 10 cm dish, 2 ml for a T25 flask, and 5 ml for a T75 flask) Rock the plate to make sure the cells are equally covered with the solution. Trypsinize the cells at room temperature for ~ 5 min. After 5 min, check the cells to ensure that they are coming off the dish/flask. Tap the dish/flask gently to aid in the process. Add enough serum-containing medium to give a volume of ~ 10 ml, and pipette the medium up and down through a 10 ml serological pipette ~ 4 times to obtain a single cell suspension. Remove a portion of the sample for a cell count.

Note. Trypsin is required to dissociate the cells during the process of passage. Cells may not be able to recover to an optimal stage if trypsin-free dissociation buffer is used.

3. For primary expansion from a frozen vial, reseed the total cell volume in a T150 flask. For routine cell passage, split the cells using a ratio of 1:4 – 1:10.
4. Observe the cells daily and harvest the cells when they reach 80-90% confluence (1-3 days). Cells do not need to be fed during this time. Do not allow the cells to grow over 90% confluence.

FREEZING AND STORING THE CELLS

1. Remove cells from T75 flask by trypsinization as described above. Add 10 ml culture medium, and break the cell clumps via pipetting. Count cells using a hemocytometer.
2. Place cell suspension in a sterile centrifuge tube, and pellet the cells at ~ 200X g at 4°C for 5 min. Remove the medium, and resuspend the cell pellet in an appropriate volume of freezing medium (90% FBS and 10% DMSO) to give a cell density of 2.5 X 10⁶ cells/ml.
3. Dispense the cells in 1 ml aliquots into cryo storage vials to give 2.5 X 10⁶ cells/vial.
4. Freeze the cells in a cryo freezing-container overnight at -80°C.
5. Next day, transfer the cell vials to a liquid nitrogen tank for long-term storage.

cAMP ASSAY PROTOCOL

Note. Please finish reading the whole protocol before you start the experiment.

CELL PREPARATION

1. Harvest cells when they reach 80-90% confluence in flasks. Trypsinize cells as described in Cell Culture Protocol above. Count a portion of the cells with a hemocytometer.

Note. It is very important that the cells DO NOT reach >90% confluence. Over-confluent growth can result in a significantly reduced response to ligands, and it may take several passages for the cells to recover to optimal stage.

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- The cell number needs to be optimized for each assay. Optimal assay conditions require a confluent monolayer of cells prior to the assay. Poly-D-Lysine coated plates are recommended to improve cell attachment. Plate 56K cells/well for a 96 well plate and 14K cells/well for a 384 well plate the day before the experiment. Add 80 μL /well of cell suspension to 96-well plates or 20 μL /well to 384-well plates.
- Allow cells to attach by leaving the cell plates at room temperature for 30 minutes. Transfer the plates to a cell culture incubator and grow the cells overnight.

PREPARATION of COMPOUND PLATES

Dilute 1 $\mu\text{g}/\mu\text{L}$ TSH stock in DPBS containing 125 μM of Ro 20-1724 as shown in Table 1. These concentrations are 5X the expected final testing concentrations.

	1	2	3	4	5	6	7	8	9	10	11	12
A	5,000	1,500	500	150	50	15	5	1.5	0.5	0.15	0.05	0

----- TSH (ng/ml) -----

Table 1. An example of TSH concentrations in a compound dilution plate

COMPOUND STIMULATION

Take the cell plate out from the incubator. Add different concentrations of TSH prepared above, 20 μL /well into a 96-well plate and 5 μL /well into a 384-well plate. Incubate at room temperature for 20 min.

The final concentrations of TSH used are listed in Table 2. (The final concentration of Ro 20-1724 is 25 μM)

	1	2	3	4	5	6	7	8	9	10	11	12
A	1,000	300	100	30	10	3	1	0.3	0.1	0.03	0.01	0

----- TSH (ng/ml) -----

Table 2. An example of the final testing concentrations of TSH in a cell assay plate

If an antagonist assay is performed, the concentrations listed below must be adjusted to accommodate the volume of antagonist added.

Dilute antagonists in DPBS to a desired concentration. Add the antagonists to the cell plate. Incubate the plates at room temperature for approximately 15 min prior to addition of the specific agonists.

Note. Antagonist incubation times should be optimized for each assay.

cAMP ELISA ASSAY

Aspirate off cell solution after the incubation with compounds. Add 100 μL /well of Cell Lysis Buffer into a 96-well plate or 25 μL /well of Cell Lysis Buffer into a 384-well plate, incubate at room temperature for another 10 minutes.

Transfer 18 μL of the cell lysate into each well of the anti-cAMP Ab coated 384-well plate. Incubate at room temperature for 5 to 10 minutes.

Add 6 μL /well of 1X HRP-cAMP conjugate working solution. Incubate at room temperature for 2 hours by placing the plate on shaker.

Aspirate plate contents, and wash the plate 4 times with 20 μL /well of 1X wash solution.

Prepare Amplite™ Red working solution by adding 100 μL of 100 X Amplite™ Red stock solution and 11.5 μL of 3% H_2O_2 into 10 mL of Substrate Buffer.

Note: The Amplite™ Red working solution is not stable, use it promptly.

Add 20 μL /well of Amplite™ Red working solution (from Step 3.5) into each well, and incubate at room temperature for 30 minutes to 1 hour.

Monitor the fluorescence intensity at Ex/Em = 540/590 nm (cutoff 570 nm) by using a fluorescence plate reader (top read mode).

APPENDIX

CELL LINE DESIGNATION

Thyroid Stimulating Hormone Receptor cell line
(CB-80200-242b)

ORIGIN (PARENTAL CELL)

HEK293

GENE INTRODUCED

Genbank Locus ID 7253

RECEPTOR INTRODUCED:

Human Thyroid Stimulating Hormone Receptor (NCBI protein
database AAR07906)

USAGE

- cAMP assay for Gs-coupled human Thyroid Stimulating Hormone Receptor (TSHR).
- HEK293 cells without transfected Thyroid Stimulating Hormone Receptor are used as a negative control.

QUALITY CONTROL

1. This cell line has been tested negative for *Mycoplasma sp.*
2. This cell line has been tested positive for Thyroid Stimulating Hormone Receptor specific response.
3. Surviving rate: More than 2.5 million/vial on the second day after thawing.

CELL CULTURE CONDITION

1. Growth medium: 90% DMEM, 10% FBS and 1 µg/ml puromycin
2. Freezing medium: 10% DMSO, 90% FBS

DATA EXAMPLE

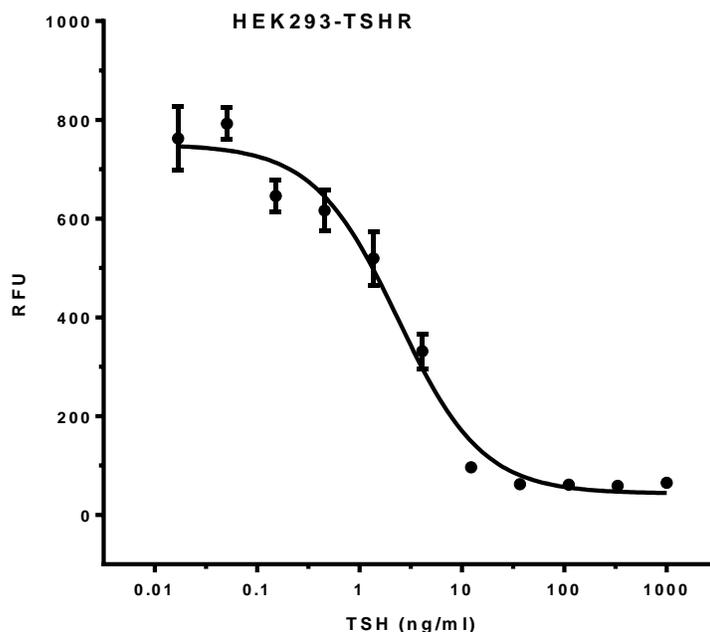


Figure 1. Response of HEK293-TSHR cell line to TSH.

TSHR cells were plated overnight in 80 µl culture medium on a 96-well plate black/clear plate. The next day, cells were treated with different concentration of TSH for 20 min in the presence of 25 µM Ro 20-1724. The cells were lysed with 100 µl of lysis buffer. 18 µl of the cell lysate were transferred into each well of a 384-well assay plate. cAMP concentration was determined with Codex-cAMP ELISA kit.

Dose response curve of TSH in HEK293-TSHR cell line. EC50 = 2.2 ng/ml (68.5 pM) in the presence of PDE inhibitor Ro20-1724.

TROUBLESHOOTING GUIDE

1. Low survival rate of cells after thawing

- Cell vials could have thawed accidentally. Store cell vials in liquid nitrogen immediately after receiving and keep frozen at all times.
- Leaving the vial at 37°C for too long during thawing will lower the survival rate. Place the vial at 37°C until cells are just thawed.
- Handle the cells gently. Don't tap the vial or pipette the cells too many times before plating the cells.
- Replace the medium four hours after thawing or when the cells have settled to remove DMSO.

2. Slow growth rate of cells

- Do not split cells before they have completely recovered from thawing and reach at least 50% confluence.
- Do not dilute cells excessively while splitting.
- Split cells before they reach 80 – 90% confluence.
- Use Trypsin-EDTA solution to dissociate cells.
- Cells may not be able to recover to an optimal stage if trypsin- free dissociation buffer is used.

3. Response to agonist is lower than expected

- Check the overall health of cells.
- Cell density is too high or too low. Cell number titration may be necessary.
- Keep cells growing in medium with proper drug selection.
- Check settings of fluorescence readers.

4. High well-to-well variations.

- Cells should be evenly distributed among wells. Before plating, microscopically examine the culture to be sure that they have been dissociated into single cells. Leave the cell plates at room temperature for 30 minutes prior to transferring the plates to a cell culture incubator.
- Check the liquid handling system for dispensing accuracy. Optimize the settings of liquid handling system so that cell monolayer is not disturbed by dye and compound addition.
- Check settings of fluorescence readers.

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