Human FSHR Stable Cell Line-HEK293

Cat. No. CSC-RG0010 **Lot. No.** (See product label)

SPECIFICATION

Species	Human
Source	HEK293
Cell Line Description	HEK293-HuFSHR cell line is a hypotriploid human cell line, which has been transfected with a Human Follicle Stimulating Hormone Receptor(FSHR) to allow stably express of the human FSHR. It is an example of a cell line transfected using our proprietary CBTGS gene screening and amplification system.
Background	Follicle stimulating hormone receptor belongs to a family of G-protein coupled receptors which activate adenylate cyclase. FSHR is a transmembrane receptor that interacts with the follicle stimulating hormone(FSH). In the ovary, the FSH receptor is necessary for follicular development and it is expressed on the granulosa cells. In the male, the FSH receptor has been identified on the Sertoli cells that are critical for spermatogenesis. Mutations in this gene cause ovarian dysgenesis type 1, and also ovarian hyperstimulation syndrome.
Growth Properties	Adherent
Morphology	Epithelial
Host Cell	HEK293
Cell Line Validation	1. Gene expression: RT-PCR experiments determined specific expression of human FSHR.2. Functional validation: FSH dose response curve in cAMP assay

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

Sub-type	Follicle Stimulating Hormone
Propagation	Complete growth medium: EMEM (EBSS), 2mM Glutamine, 1% Non Essential Amino Acids (NEAA), 10% FCSAtmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37.0°C
Starting Cells From Frozen Cell Stock	1. Prepare a poly-L-lysine coated flask (2µg/cm2, T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 15 µl of poly-Llysine stock solution (10 mg/ml). Leave the flask in incubator overnight (minimum one hour at 37°C incubator). 2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer themto sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.3. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.4. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Centrifuge at 125xg for 5 min to collect the cells Remove the cap, being careful not to touch the interior threads with fingers. Remove the frozeen media using 1 ml eppendorf pipette and gently resuspend the contents of the vial in 1ml of pre-warmed complete growth media.5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels containing 9ml prewarmed complete growth media. A seeding density of 3 x 10^6 cells/cm2 is recommended. 6. Place the culture vessels to the incubator.7. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter. A healthy culture will display stellate or spindle-shaped cell morphology, nongranular cytoplasm, and the cell number will be double after two to three days in culture.

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

Subculturing	1. Subculture the cells when they are over 90% confluent.2. Prepare poly-L-lysine coated cell culture flasks.3. Warm medium, trypsin/EDTA solution, trypsin neutralization solution, and DPBS to room temperature. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.4. Rinse the cells with DPBS.5.Incubate cells with 5 ml of trypsin/EDTA solution (in the case of T-75 flask) until 80% of cells are rounded up (monitored with microscope). Add 5ml of trypsin neutralization solution to the digestion immediately and gently rock the culture vessel. 6. Harvest and transfer released cells into a 50 ml centrifuge tube. Rinse the flask with another 10 ml of growth medium to collect the residue cells. Examine the flask under microscope to make sure the harvesting is successful by looking at the number of cells left behind. There should be less than 5%. 7. Centrifuge the harvested cell suspension at 1000 rpm for 5 min and resuspend cells in growth medium.8. Count cells and plate them in a new, poly-L-lysine coated flask with cell density as recommended.
Mycoplasma	Mycoplasma Status: Negative (MycoAlert Kit)
Freeze Medium	Complete growth medium 90%; DMSO, 10%
Storage	Liquid nitrogen
Preservation	1. Detach cells from culture dish according to the Sub-Culture Procedure.2. Resuspend cells at a density of 5 x 10^6 cells/mL in freeze medium.Note: A T-75 culture flask typically yields enough cells for preparing two frozen vials.3. Aliquot 1 mL cells into cryogenic vials.4. Place vials in a freezing container and store at –80 °C overnight.5. Transfer vials to liquid nitrogen for long term storage. If properly stored, cells should remain stable for years.
Safety Considerations	The following safety precautions should be observed.1. Use pipette aids to prevent ingestion and keep aerosols down to a minimum.2. No eating, drinking or smoking

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

	while handling the stable line.3. Wash hands after handling the stable line and before leaving the lab.4. Decontaminate work surface with disinfectant or 70% ethanol before and after working with stable cells.5. All waste should be considered hazardous.6. Dispose of all liquid waste after each experiment and treat with bleach.
Ship	Dry ice

GENE INFORMATION

Gene Name	FSHR follicle stimulating hormone receptor [Homo sapiens]
Official Symbol	FSHR
Synonyms	FSHR; follicle stimulating hormone receptor; ODG1; follicle-stimulating hormone receptor; FSHRO; LGR1; FSH receptor; follitropin receptor; MGC141667; MGC141668;
Gene ID	2492
mRNA Refseq	NM_000145
Protein Refseq	NP_000136
MIM	136435
UniProt ID	P23945
Chromosome Location	2p21-p16
Pathway	Class A/1 (Rhodopsin-like receptors), organism-specific biosystem; G alpha (s)

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

	signalling events, organism-specific biosystem; GPCR downstream signaling, organism-specific biosystem; GPCR ligand binding, organism-specific biosystem; GPCRs, Class A Rhodopsin-like, organism-specific biosystem; GPCRs, Other, organism-specific biosystem; Hormone ligand-binding receptors, organism-specific biosystem;
Function	follicle-stimulating hormone receptor activity; peptide hormone binding; protein binding; receptor activity; signal transducer activity;

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA