CHO/Human GPRC5D Stable Cell Line Data Sheet

CHO/Human GPRC5D Stable Cell Line

Catalog No.	Clone No.	Size
CCHO-STP078	ACH1348C1	1 vial containing at least 5x10^6 cells

• *Description*

CHO/Human GPRC5D Stable Cell Line is a stably transfected CHO cell line which expresses full length human GPRC5D (Accession # Q9NZD1-2). Surface expression of GPRC5D was confirmed by flow cytometry.

• *Cell Line Profile*

Cell line	CHO/Human GPRC5D Stable Cell Line
Species	Human
Property	Suspension
Medium	OPM-CHO CDP3 medium
Selection Marker	MSX (20 μM)
Incubation	37℃ with 5% CO_2
Storage	Frozen in liquid nitrogen
Biosafety Level	1
Application	Binding assay by FACS and cell based ELISA.

• *Materials Required for Cell Cultur*

- OPM-CHO CDP3 medium (OPM, Cat.No. P081863-001)
- MSX (SIGMA, Cat.No. M5379-250MG)
- Anti-Clumping Additive (Acro, Cat.No. AC-1112-11-100ml)
- Fetal bovine serum (CellMax, Cat.No.SA211.01)
- DMSO (Applichem, Cat.No. A3672,0250)
- 90mm-culture dishes (SARSTEDT, Cat.No.83-3902)
- Cryogenic storage vials (greiner, Cat.No.122280)
- Thermostat water bath
- Centrifuge
- Luna cell counter (Cellaca, MX)
- Stacked thermostatic oscillator / Superimposed thermostatic oscillator (CTI, IS-9C5)
- Biological Safety Cabinet (HDL, BSC-1360IIA2)

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• Recovery

a. Rapidly thaw (< 2 minute) frozen vial of cell in a 37℃-water bath.

b. Transfer the cell suspension into a tube with 5 mL OPM-CHO CDP3 medium.

c. Appropriate amount of cell fluid was taken for counting.

d. Spin down the cells at 200 × g for 5 minutes.

e. Resuspend cell pellet with appropriate volume of OPM-CHO CDP3 medium and transfer the cell suspension into tube or shake flask. The cell culture density is 0.5-0.6 ×10^6 cells/mL .

f. Incubate the cells in a 37 ℃ incubator and 5% CO_2 on an orbital shake platform.

Note：Set the shake speed to 120 ± 5 rpm for shakers with 25mm shaking diameter.

• Subculture

a. Viability may be poor on resuscitation, full recovery may take up to a week. 2-3 days later, Appropriate amount of cell fluid was taken for counting.

b. Using the viable cell density, calculate the volume of cell suspension required to seed a new shake flask according to the recommended seeding densities. For cells ready 3 days post-subculture, recommended seeding density are 0.3 × 10^6–0.4 × 10^6 viable cells/mL ； for cells ready 2 days post-subculture, recommended seeding density are 0.5 × 10^6–0.6 × 10^6 viable cells/mL .

c. Transfer the calculated volume of cells to fresh, pre-warmed OPM-CHO CDP3 medium in a shake flask.

d. Incubate flasks in a 37°C incubator and 5% CO2 on an orbital shaker platform until cultures reach a density of 2 × 10^6 – 3 × 10^6 viable cells/mL.

*Note: Add **20 μM MSX** from first subculture. Add appropriate Anti-Clumping Agent if the cells occur clumping phenomenon.*

• Cryopreservation

a. The best freezing time is the second week after resuscitation. Freeze the cells at a final density between 5 × 10^6 and 2 × 10^7 viable cells/mL.

b. Use a freezing medium composed of 90% FBS and 10% DMSO.

Note: Check the viability and recovery of frozen cells 24 hours after storing cryovials in liquid nitrogen by following the procedure outlined in Recovery.