

HEK293/Human NPR1 Stable Cell Line Data Sheet

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ACKNOWLEDGMENT

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HEK293/Human NPR1 Stable Cell Line Data Sheet

HEK293/Human NPR1 Stable Cell Line

| Catalog No. | Size |
|-------------|--|
| CHEK-ATP245 | 2 × (1 vial contains ~5×10 ⁶ cells) |

• Description

The HEK293/Human NPR1 Stable Cell Line was engineered to express the receptor full length human NPR1 (Uniprot: P16066). Surface expression of human NPR1 was confirmed by flow cytometry.

• Application

- Useful for cell-based NPR1 binding assay
- Screen for human NPR1 agonists based on cGMP accumulation assay

• Cell Line Profile

| | |
|------------------------|------------------------------------|
| Cell line | HEK293/Human NPR1 Stable Cell Line |
| Host Cell | HEK293 |
| Property | Adherent |
| Complete Growth Medium | DMEM + 10% FBS |
| Selection Marker | Hygromycin B (20 µg/mL) |
| Incubation | 37°C with 5% CO ₂ |
| Doubling Time | 22-24 hours |
| Transduction Technique | Lentivirus |

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• *Materials Required for Cell Culture*

- DMEM Medium (BasalMedia, Cat. No. L120KJ)

Note: If you are unable to obtain the specified DMEM medium (BasalMedia, Cat. No. L120KJ), you may use an alternative DMEM medium (Gibco, Cat. No. 11965-092) or another suitable medium for culturing.

- Fetal bovine serum (CellMax, Cat. No. SA211.02)
- Hygromycin B (Invitrogen, Cat. No. 10687010)

Note: For selection antibiotics, we highly recommend using the specified brand. The activity of antibiotics may vary between manufacturers, so if you choose to use a different brand, it is essential to validate whether the concentration recommended in the culture medium is suitable. Regardless of the brand used, we recommend maintaining a backup culture without selection antibiotics to avoid potential cell loss due to inappropriate antibiotic concentration.

- 0.25% Trypsin-EDTA (1X), Phenol Red (Gibco, Cat. No. 25200-056)
- Penicillin-Streptomycin (Gibco, Cat. No. 15140-122)
- Phosphate Buffered Saline (1X) (HyClone, Cat. No. SH30256.01)
- Complete Growth Medium: DMEM + 10% FBS, 1%P/S
- Culture Medium: DMEM + 10% FBS, Hygromycin B (20 µg/mL), 1%P/S
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, Cat. No. 430641)
- Cryogenic storage vials (SARSTEDT, Cat. No. 72.379.007)
- Thermostat water bath
- Centrifuge (Cence, Model: L550)
- Cell counter (MONWEI, Model: SmartCell200A Plus)
- CO₂ Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

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• *Recovery*

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize the risk of contamination, ensure the cap remains out of the water. Thawing should be completed quickly, typically within 3-5 minutes.
2. After thawing, promptly remove the vial from the water bath and decontaminate it by spraying with 70% ethanol. From this point onward, all operations must be performed under strict aseptic conditions.
3. Transfer the contents of the vial to a centrifuge tube containing 4.0 mL of complete growth medium. Centrifuge at approximately 1000 rpm for 5 minutes.
4. Resuspend the cell pellet with 5 mL **complete growth medium** and transfer the cell suspension into a T-75 flask containing 10-15 mL of pre-warmed **complete growth medium**.
5. Incubate at 37°C with 5% CO₂ incubator until the cells are ready to be split.

• *Subculture*

1. Cell viability may be low after thawing, and full recovery may take up to a week. Monitor the cells daily until the culture reaches 80-90% confluency. At this point, remove and discard the spent medium. Avoid allowing the cells to become over-confluent to ensure optimal cell health.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of **culture medium** using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps.
5. Transfer appropriate aliquots of the cell suspension to a new T-75 flask. A subcultivation ratio of 1:4 to 1:8 is recommended. Adjust the ratio based on your specific culture system.
6. Incubate at 37°C with 5% CO₂ incubator.
7. When the cell culture reaches 80-90% confluency, proceed to the next subculture. Avoid over-confluency, as this may negatively impact cell performance in subsequent passages.

Note: After recovery, maintain the cells for 1-2 passages in the complete growth medium not containing the selection marker, if the cells are in good condition, transition to the culture medium containing the selection marker during subculturing.

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• *Cryopreservation*

1. When the cell culture reaches 80-90% confluency, remove and discard the spent medium.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of complete growth medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps. Count the viable cells.
5. Transfer the cell suspension to a centrifuge tube. Centrifuge at 1000 rpm for 5 min at room temperature to pellet the cells.
6. After centrifugation, discard the supernatant. Resuspend the cells in ice cold freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
7. Aliquot the cell suspension into cryogenic storage vials. Place the vials in a programmable cooler or an insulated box placed in a -80°C freezer overnight, then transfer to liquid nitrogen storage for long-term storage.

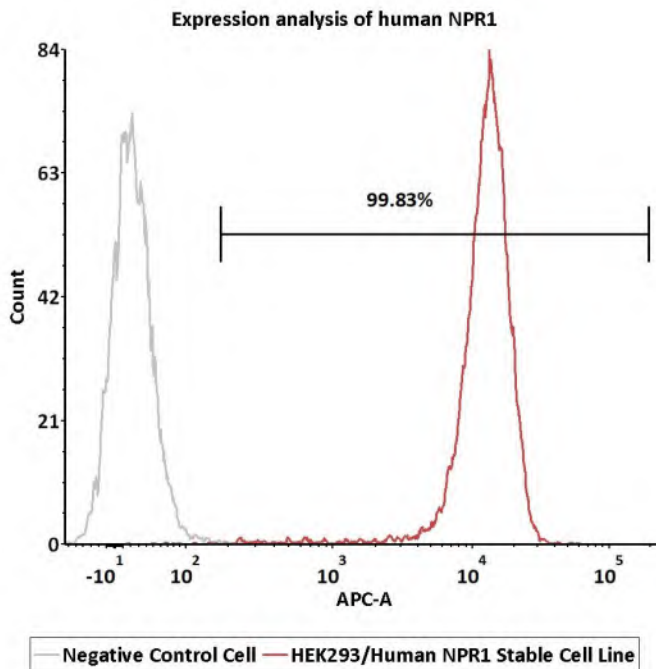
Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *Storage Condition*

Cells must be received in a frozen state on dry ice and should be transferred to liquid nitrogen or a -80°C freezer immediately upon receipt. If stored in a -80°C freezer, it is recommended to limit the storage period to no more than two weeks. For long-term preservation, transfer the cells to liquid nitrogen is highly recommended.

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• *Receptor Assay*



| Catalog No. | Stable Cell Line | MFI for NPR1 (APC) |
|--------------------|---|--------------------|
| NA | Negative Control Cell | 17.99 |
| CHEK-ATP245 | HEK293/Human NPR1 Stable Cell Line | 12605.03 |

Fig1. Expression analysis of human NPR1 on HEK293/Human NPR1 Stable Cell Line by FACS. Cell surface staining was performed on HEK293/Human NPR1 Stable Cell Line or negative control cell using anti-human NPR1 antibody followed by staining with APC anti-human IgG antibody.

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• Signaling Bioassay

Human ANP Stimulation

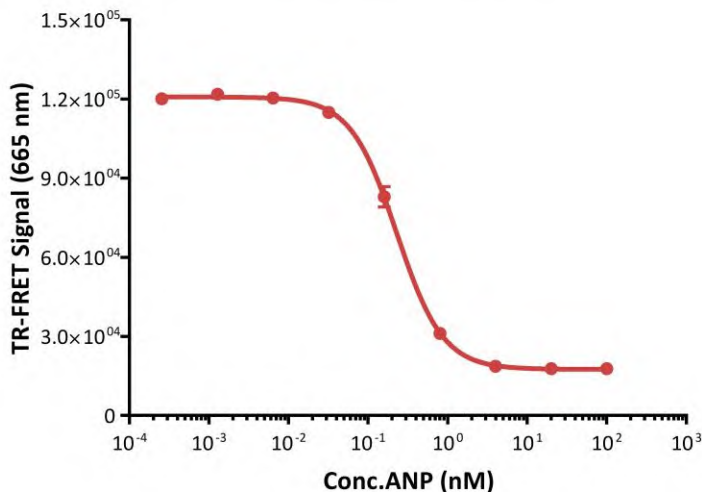


Fig2. Response to human ANP measured by cGMP accumulation assay. The HEK293/Human NPR1 Stable Cell Line were stimulated with serial dilutions of human ANP. The EC₅₀ of human ANP was approximately 0.2292 nM.

Human BNP Stimulation

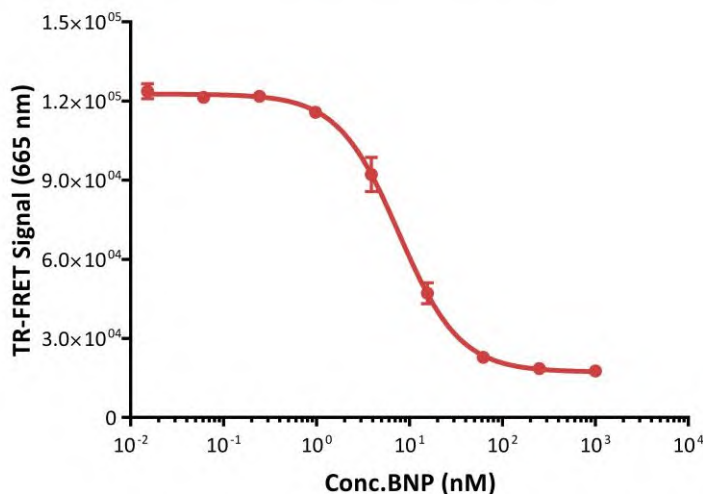


Fig3. Response to human BNP measured by cGMP accumulation assay. The HEK293/Human NPR1 Stable Cell Line were stimulated with serial dilutions of human BNP. The EC₅₀ of human BNP was approximately 7.684 nM.

HEK293/Human NPR1 Stable Cell Line Data Sheet

• *Related Products*

| <u>Products</u> | <u>Cat.No.</u> |
|---|-----------------------|
| HEK293/hClaudin-18.2 Cell Line | CHEK-ATP033 |
| HEK293/hGPRC5D Cell Line | CHEK-STP042 |
| HEK293/Human TROP-2 Stable Cell Line | CHEK-ATP036 |
| HEK293/Human Nectin-4 Stable Cell Line | CHEK-ATP035 |
| HEK293/Human Anti-CD19 Stable Cell Line | CHEK-ATS056 |
| CHO/Human GPRC5D Stable Cell Line | CCHO-STP078 |
| HEK293/Human CEACAM5 Stable Cell Line | CHEK-ATP083 |
| HEK293/Human ROR1 Stable Cell Line | CHEK-ATP084 |
| HEK293/Human Transferrin R Stable Cell Line | CHEK-ATP089 |
| HEK293/Human DLL3 Stable Cell Line | CHEK-ATP090 |
| HEK293/Human FOLR1 Stable Cell Line | CHEK-ATP091 |
| HEK293/Human Glypican-3 (GPC3) Stable Cell Line | CHEK-ATP092 |
| CHO/Human DLL3 Stable Cell Line | SCCHO-ATP111 |
| CHO/Human Glypican-3 (GPC3) Stable Cell Line | SCCHO-ATP112 |
| HEK293/Human Transferrin Stable Cell Line | CHEK-ATP115 |
| HEK293/Human NAPI-IIb Stable Cell Line | CHEK-ATP116 |
| HEK293/Human Mesothelin Stable Cell Line | CHEK-ATP119 |
| CHO/Human Mesothelin Stable Cell Line | SCCHO-ATP120 |
| CHO/Human STEAP1 Stable Cell Line | SCCHO-ATP121 |
| HEK293/Human ENPP3 Stable Cell Line | CHEK-ATP122 |
| HEK293/Human LRRC15 Stable Cell Line | CHEK-ATP123 |
| HEK293/Human Claudin-1 Stable Cell Line | CHEK-ATP124 |
| HEK293/Human Integrin alpha V beta 6 Stable Cell Line | CHEK-ATP125 |
| HEK293/Human B7-H4 Stable Cell Line | CHEK-ATP126 |
| HEK293/Human Cadherin-6 Stable Cell Line | CHEK-ATP127 |
| HEK293/Human LY6G6D Stable Cell Line | CHEK-ATP137 |
| HEK293/Human Claudin-6 Stable Cell Line | CHEK-ATP138 |
| HEK293/Human Claudin-9 Stable Cell Line | CHEK-ATP139 |

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• *Related Products*

| <u>Products</u> | <u>Cat.No.</u> |
|---|----------------|
| CHO/Human c-MET Stable Cell Line | SCCHO-ATP141 |
| HEK293/Human c-MET Stable Cell Line | CHEK-ATP146 |
| HEK293/Human EGF R Stable Cell Line | CHEK-ATP148 |
| HEK293/Human ErbB3 Stable Cell Line | CHEK-ATP149 |
| HEK293/Human ErbB2 Stable Cell Line | CHEK-ATP150 |
| HEK293/Human uPAR Stable Cell Line | CHEK-ATP151 |
| CHO/Human uPAR Stable Cell Line | SCCHO-ATP152 |
| HEK293/Human CD19 Stable Cell Line | CHEK-ATP003 |
| HEK293/Human STEAP1 Stable Cell Line | CHEK-ATP154 |
| CHO/Human CD79A&CD79B Stable Cell Line | SCCHO-ATP170 |
| CHO/Human CD79B Stable Cell Line | SCCHO-ATP171 |
| HEK293/Human Cadherin-17 Stable Cell Line | CHEK-ATP173 |
| HEK293/Human EpCAM Stable Cell Line | CHEK-ATP175 |
| HEK293/Human TPBG Stable Cell Line | CHEK-ATP176 |
| CHO/Cynomolgus Glypican-3 (GPC3) Stable Cell Line | SCCHO-ATP179 |
| HEK293/Human GUCY2C Stable Cell Line | CHEK-ATP182 |
| HEK293/Human SEZ6 Stable Cell Line | CHEK-ATP183 |
| HEK293/Human FAP Stable Cell Line | CHEK-ATP184 |
| HEK293/Human PSMA Stable Cell Line | CHEK-ATP185 |
| HEK293/Human PTK7 Stable Cell Line | CHEK-ATP186 |
| HEK293/Human MCAM Stable Cell Line | CHEK-ATP195 |
| HEK293/Human GPC3 ΔHS Stable Cell Line | CHEK-ATP212 |
| HEK293/Human c-MET&ErbB3 Stable Cell Line | CHEK-ATP217 |
| HEK293/Human BCMA Stable Cell Line | CHEK-ATP218 |
| CHO/Human CD32a (131R) Stable Cell Line | SCCHO-ATP223 |
| CHO/Human CD16a (158F) Stable Cell Line | SCCHO-ATP224 |
| CHO/Human CD89 Stable Cell Line | SCCHO-ATP225 |
| Raji/Human TL1A Stable Cell Line | CRAJ-STP232 |

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• *Related Products*

| <u>Products</u> | <u>Cat.No.</u> |
|---|----------------|
| CHO/Human CDCP1 (R368A, K369A) Stable Cell Line | CCHO-ATP234 |
| CHO/Human CDCP1 (NTF&CTF) Stable Cell Line | CCHO-ATP235 |
| HEK293/Human Tissue Factor Stable Cell Line | CHEK-ATP240 |
| CHO/Human MAdCAM-1 Stable Cell Line | CCHO-ATP241 |
| HEK293/Human Integrin alpha 4 beta 7 (ITGA4&ITGB7) Stable Cell Line | CHEK-ATP243 |
| Jurkat/Human Transmembrane TNF-alpha (mTNF) Stable Cell Line | CJUR-STF248 |
| CHO/Human CXCR3 Stable Cell Line | CCHO-ATP252 |
| HEK293/Human CXCR3 Stable Cell Line | CHEK-ATP253 |
| CHO/Human Integrin alpha V beta 6 Stable Cell Line | CCHO-ATP254 |
| HEK293/Human ASGR2 Stable Cell Line | CHEK-ATP256 |
| Jurkat/Luc Stable Cell Line | CJUR-STP258 |
| HEK293/Human FGF R2 (IIIb) Stable Cell Line | CHEK-ATP259 |
| HEK293/Human STEAP2 Stable Cell Line | CHEK-ATP263 |
| CHO/Human PD-1 Stable Cell Line | CCHO-ATP266 |