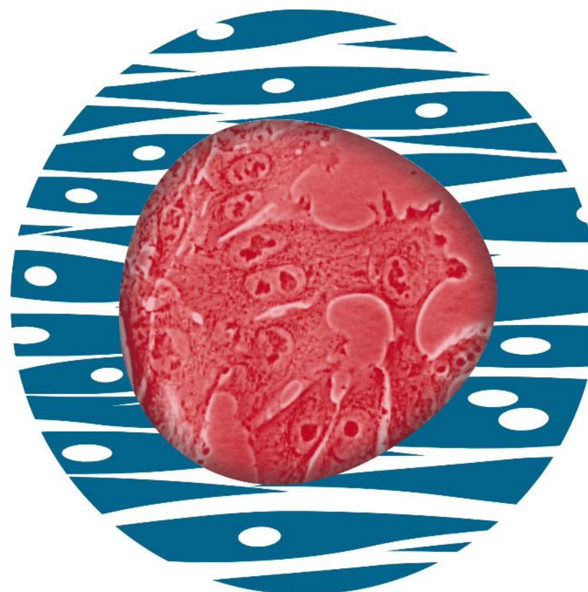
HEK293-PSMA

Cat.-No.: INS-SF-1047

PSMA Expressing Stable Recombinant HEK293 Cell Line

Product Sheet

→ At a glance		
BSL Level 1	**Coating** Collagen Coating (INS-SU-1017) for initial recovery	**Growth** Adherent
Storage <2d: -80°C >2d: liquid Nitrogen	**Medium** HEK293 Growth Medium C (INS-ME-1046)	**Expression Level** Not determined



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→ Intended Use and Licensing

This product is intended for in vitro laboratory research use only. It is not intended for any animal or human therapeutic or diagnostic use.

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→ Background Information

HEK293-PSMA is a recombinant HEK293 cell line expressing full length human PSMA (Prostate-Specific Membrane Antigen).

Catalog number: INS-SF-1047

Target: human PSMA

Target aliases: FOLH1, NAALAD1, GCPII, Glutamate Carboxypeptidase 2

Target expression level(s): n. d.

Biosafety level (BSL): Level 1

Cell Background: HEK293 (*Homo sapiens*, human)

Growth properties: adherent

Target Background

Type II transmembrane glycoprotein of the M28 peptidase family with folate hydrolase and N-acetylated-alpha-linked-acidic dipeptidase (NAALADase) activity, with preference for tri-alpha-glutamate peptides. Acts as a glutamate carboxypeptidase on substrates including dietary folates and the neuropeptide N-acetyl-L-aspartyl-L-glutamate (NAAG). In the intestine, it is required for folate uptake; in the brain, it hydrolyzes NAAG and releases glutamate, and brain expression may be involved in conditions linked to glutamate excitotoxicity. Expressed in multiple tissues including prostate, nervous system, kidney, and small intestine. Upregulated in prostate cancer and used as a diagnostic and prognostic indicator. Alternative splicing yields multiple isoforms.

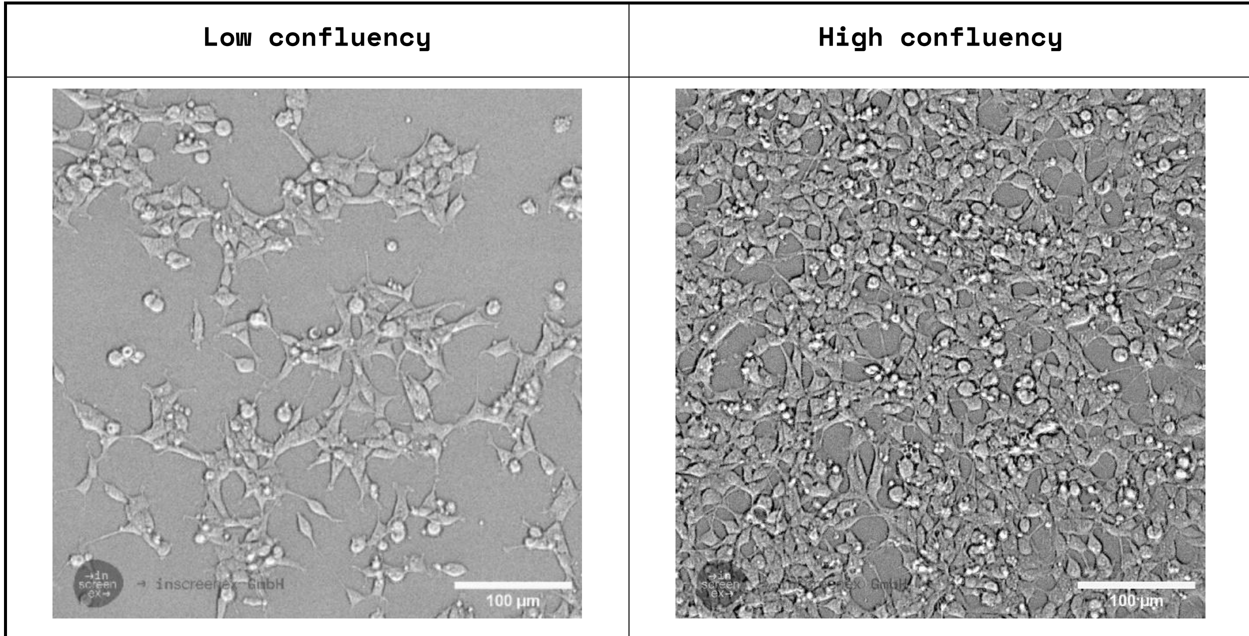
Note	*All target sequences undergo codon optimization and other sequence modifications on DNA level to improve recombinant expression and the nucleotide sequence of the recombinant protein therefore differs from database reference sequences. For details refer to section* *Target Sequence on page 9*.

Cell Line Generation

This cell line was generated using our **in**screenex landing pad cell lines. These cells contain a recombination site and a selectable marker at a pre-validated genomic locus. Using a matching recombinase and specifically designed expression setups, the DNA payload, i.e. the target, is then specifically inserted into that locus, allowing for reproducible integration at well-defined sites in the genome. This significantly reduces the effort and timelines to isolate a stable clonal population. Expression of the target was then analyzed using flow cytometry and target-specific antibodies.

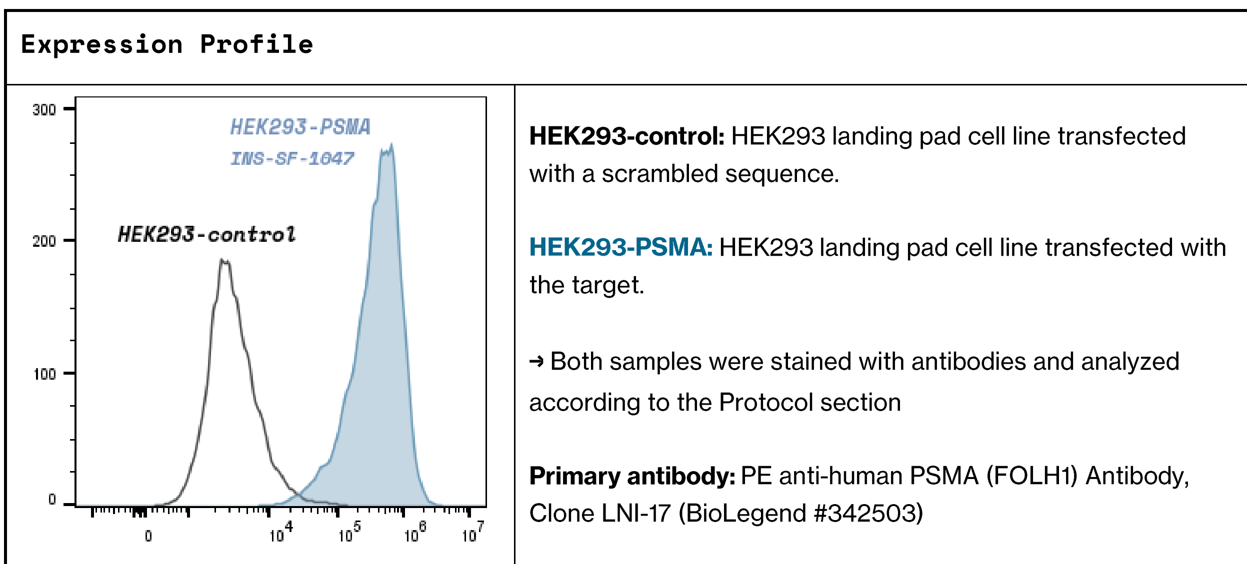
→ Morphology

adherent; epithelial-like; grows in monolayer



→ Cell Characterization

Target expression was analyzed using a target-specific antibody and the indicated staining protocol.



Materials	Protocol
– PBS/EDTA solution – 2% FBS/FCS in PBS (FACS Buffer) – Primary antibody: PE anti-human PSMA (FOLH1) Antibody, Clone LNI-17 (BioLegend #342503)	**Wash Protocol:** Add FACS Buffer, resuspend cells gently, then centrifuge at 300×g for 5min. 1) Prepare detection reagents in FACS buffer. 2) Aspirate medium from cells. 3) Add PBS/EDTA solution to the cells and incubate at room temperature or 37°C for 5-10min, or until the cells detach. 4) Wash cells 1×. 5) Add primary antibody in FACS buffer, resuspend cells gently. 6) Incubate at ambient temperature for 20-30min. 7) Wash cells 2×. 8) Resuspend in 100-200µl FACS Buffer. 9) Analyze cells using a flow cytometer.

→ Quality Control

Basic information on quality control can be found below. For more details, request a Certificate of Analysis (CoA) by emailing info@inscreenex.com and stating your Lot number.

Cell number: >0.5Mio viable cells (see info on vial label for exact cell number)

Viability: >75% post-thaw viability

Sterility: no contamination detected

Mycoplasma: no contamination detected

Human pathogens: Host cell line negative for HIV-1/2, HBV, HCV

→ Related Products

Products that are related to the HEK293-PSMA cell line and are either required or recommended for a successful cell culture.

Required	Recommended
***Medium*:** HEK293 Growth Medium C (INS-ME-1046) ***Coating solution*:** Collagen Coating (INS-SU-1017) for best attachment after thawing.	***Cryopreservation*:** Cell Freezing Medium (INS-SU-1027)

→ Upon Arrival

Cells are routinely shipped on dry ice. Check all containers for leakage and breakage. Check if cells arrived frozen.

If, immediately upon arrival…	…Contact us:
– the vial appears damaged, – the dry ice level in the shipping container appears low, – the cells appear thawed, or – you have any other concerns regarding the quality of the cells	1) take photos of the vial and/or the shipping container, 2) contact us by email or telephone (see General Inquiries on page 2).
If everything looks good, either seed the cryopreserved cells immediately, or store them:	
– at -80°C for periods of up to 2 days, or – below -130°C in liquid nitrogen vapor, for long term storage.	

→ Coating Protocol

Our Collagen Coating Solution is a sterile, ready-to-use solution ideal for coating cell culture surfaces.

Note	*For best attachment and viability after thawing the cells, we recommend using Collagen-coated flasks or plates for the initial seeding after cryo-recovery.* *Collagen coating is not required for subsequent routine culture of the cells.*

Required materials	Protocol
– Cell culture plastic (flasks, dishes, plates) – Sterile PBS – Collagen Coating (INS-SU-1017)	1) Cover the cell culture vessel with Collagen Coating (see table to the left for recommended volumes). Rock back and forth to cover the entire surface if necessary. 2) Incubate for at least 2h at 37°C in the incubator or overnight at 2–8°C. 3) Aspirate Collagen Coating. 4) Wash once with at least 4 volumes of sterile PBS. 5) Aspirate PBS. 6) Use immediately or store coated cell culture plastic according to instructions provided to the left.
Storage	
– Store Collagen Coating at 2–8°C, if not indicated otherwise on the product label. – Store coated cell culture plastic sealed at 2–8°C for up to 7 days.	

Recommended volumes	
Flask/Plate	**Coating Volume**
T75	3ml
T25	1ml
6-well	0.7ml
12-well	0.25ml
24-well	0.1ml
48-well	75µl
96-well	50µl

→ Medium Information

Note	*We provide a ready-to-use HEK293 Growth Medium C (INS-ME-1046) and Cell Freezing Medium (INS-SU-1027) for the culture and cryopreservation of stable HEK293 cells.*

Storage: Store HEK293 Growth Medium C at 4 to 8°C.

Stability: See Expiry Date on bottle label.

Preparation: Ready-to-use, no preparation required

Selection antibiotic	Anti-contamination antibiotics
Our HEK293 Growth Medium C is shipped ready to use and already contains the selection antibiotic, Hygromycin (150µg/ml), to guarantee stable long-term expression of the target.	Our HEK293 Growth Medium C does not contain prophylactic antibiotics for prevention of contamination. If you wish to use antibiotics, any standard, cell culture grade antibiotics can be added to the medium.

→ Thaw Cryopreserved Cells

Do not thaw the cells until the recommended medium and coated flasks are on hand. For initial recovery (after delivery of the cells), we recommend thawing the cells on a T25 flask and not exceeding a split ratio of 1:2 to 1:3 for the first split after thawing.

Note	*For best attachment and viability after thawing the cells, we recommend using Collagen-coated flasks or plates for the initial seeding after cryo-recovery.* *Collagen coating is not required for subsequent routine culture of the cells.*

Required materials	*Protocol*
– Coated cell culture vessel (see section Coating Protocol on page 6) – HEK293 Growth Medium C (INS-ME-1046) pre-warmed to 37°C – 15ml tube with a conical bottom suitable for centrifugation (e.g. "Falcon tube")	1) Add 4ml pre-warmed medium to a 15ml tube. 2) Quickly thaw the cryovial at 37°C in a water bath until only a few ice crystals are visible. Disinfect vial briefly by spraying with 70% Ethanol. 3) Transfer thawed cell suspension to the 15ml tube containing 4ml medium. Avoid excessively pipetting up and down. 4) Centrifuge cells at 300×g for 5min. 5) Aspirate supernatant. 6) Gently resuspend the cell pellet in complete Medium. Use a volume appropriate for the cell culture vessel. 7) Transfer cells in coated cell culture vessel and place in the incubator (37°C, 5% CO2). 8) Change the medium after 2 days.

→ Freeze Cells for Cryopreservation

Cell should be grown to 90% confluence before cryopreservation. Avoid full confluence before cryopreservation.

Required materials	*Protocol*
– Cell Freezing Medium (INS-SU-1027) – PBS – Trypsin/EDTA solution (TE) – 2% FBS in PBS – 15ml tube – Cryovial(s) – Freezing container ("Mr. Frosty" or similar) – 15ml tube with a conical bottom suitable for centrifugation (e.g. "Falcon tube")	1) Aspirate medium, wash with PBS and aspirate PBS. 2) Add Trypsin/EDTA (TE) solution to the cells and incubate at room temperature or 37°C for 5-10min, or until the cells detach. 3) Examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells. 4) Resuspend cells in 2% FBS in PBS and transfer to a 15ml conical bottom tube. 5) Centrifuge cells at 300×g for 5min. 6) Aspirate supernatant and gently resuspend cell pellet in Freezing medium (approx. 1Mio. cells/ml). 7) Transfer cell suspension into cryovial(s) and place them into a freezing container ("Mr. Frosty" or similar). 8) Place the freezing container at -80°C for 16-24h. 9) Transfer cryovials to liquid nitrogen vapor for long-term-storage.

→ Routine Adherent Cell Culture

Work in a sterile environment and follow Good Cell and Tissue Culture Practice.

Temperature: 37°C

Environment: 5% CO_2 (v/v), humidified atmosphere

Split ratio: 1:2 for initial split after thawing, 1:5 to 1:10 for routine culture

Confluence: split at 70–90% confluence

Medium change: every 2–3 days

Required materials

- HEK293 Growth Medium C (INS-ME-1046)
- PBS
- Trypsin/EDTA solution (TE)

Recommended volumes

Flask or Plate	*Medium or PBS*	*TE solution*
T75	8–10ml	3ml
T25	4–5ml	1ml
6-well	1.5–3ml	0.7ml
12-well	1–2ml	0.25ml
24-well	0.5–1ml	0.1ml
48-well	0.2–0.4ml	75µl
96-well	0.1–0.2ml	50µl

Protocol

1) Aspirate medium.
2) Wash with PBS and aspirate PBS.
3) Add Trypsin/EDTA (TE) solution to the cells and incubate at room temperature or 37°C for 5-10min, or until the cells detach.
4) Examine the cells under a microscope. When the cells start to detach, gently tap the side of the vessel to loosen the remaining cells.
5) Resuspend cells in complete Medium thereby inactivating the Trypsin/EDTA (TE) solution.
6) Transfer an aliquot of the cell suspension to a new cell culture vessel containing fresh complete Medium.
7) Place into incubator.

→ Target Sequence

Note	*All target sequences undergo codon optimization and other sequence modifications on DNA level to improve heterologous expression. While the protein (amino acid sequence) is identical to published reference sequences, the DNA and RNA sequence may therefore deviate from published reference sequences.*

Amino Acid Sequence (Protein)

Uniprot ID: *Q04609-1*

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Nucleotide sequence (DNA)

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→ References

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