



HEK293-CCR8

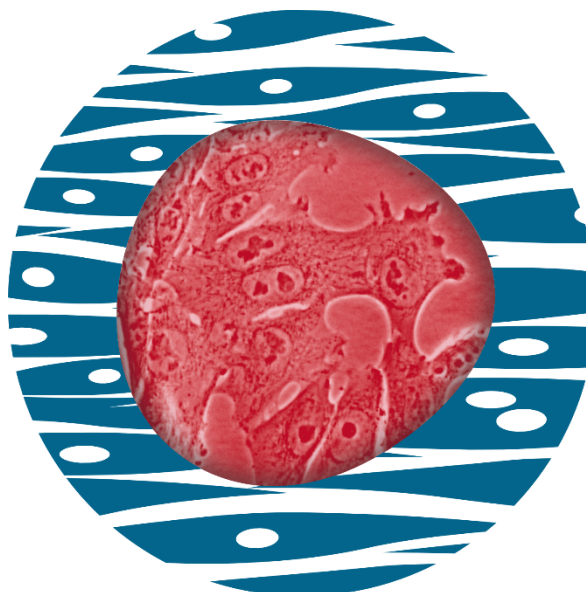
Cat.-No.: INS-SF-1024

CCR8 Expressing Stable Recombinant

HEK293 Cell Line

Product Sheet

→ At a glance		
BSL <i>Level 1</i>	Coating <i>Collagen Coating (INS-SU-1017) for initial recovery</i>	Growth <i>Adherent</i>
Storage <i><2d: -80°C >2d: liquid Nitrogen</i>	Medium <i>HEK293 Growth Medium A (INS-ME-1041)</i>	Expression Level <i>Medium-high (22000 molecules/cell)</i>





→ Contents

→ Intended Use and Licensing	2
→ Background Information	3
Target Background	3
Cell Line Generation	3
→ Morphology	4
→ Cell Characterization	4
Target Expression Level	4
Receptor Density	5
→ Quality Control	5
→ Related Products	6
→ Upon Arrival	6
→ Coating Protocol	6
→ Medium Information	8
Ready-to-use HEK293 Growth Medium A	8
→ Thaw Cryopreserved Cells	8
→ Freeze Cells for Cryopreservation	9
→ Routine Adherent Cell Culture	9
→ Target Sequence	11
Amino Acid Sequence (Protein)	11
Nucleotide sequence (DNA)	11
→ References	11

inscreenex GmbH

Inhoffenstr. 7
38124 Braunschweig
Germany

→ General Inquiries

M. info@inscreenex.com

T. +49 531 6181 5080

→ Ordering

M. orders@inscreenex.com

→ Licensing

M. licensing@inscreenex.com

→ 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.

If you have purchased this cell line for **academic, non-profit research**, the use of this cell line is governed by the **inscreenex Limited Research Use License (LRUL)**. Please refer to the LRUL for the full terms and conditions, and relevant use limitations. If you have purchased this cell line for **commercial, for-profit research**, the use of this cell line is governed by the **inscreenex Limited Commercial Use License (LCUL)**. Please refer to the LCUL for the full terms and conditions, and relevant use limitations. If you wish to use the cell line for commercial purposes that fall outside the permitted use in the LCUL please contact licensing@inscreenex.com.



→ Background Information

HEK293-CCR8 is a recombinant HEK293 cell line expressing full length human CCR8.

Catalog number: INS-SF-1024

Target: human CCR8

Target aliases: CHEMR1, CDw198, TER1, GPRCY6

Target expression level(s): Medium-high (approx. 22 000 molecules/cell)

Biosafety level (BSL): Level 1

Cell Background: HEK293 (Homo sapiens, human)

Growth properties: adherent

Target Background

C-C chemokine receptor type 8 (CCR8), a cell surface receptor within the G protein-coupled receptor (GPCR) family, is expressed on various immune cells, notably regulatory T cells (Tregs), which suppress immune responses crucial for tumor eradication. Tregs, influenced by cytokines like TGF- β and IL-2, utilize CCR8 for tumor infiltration, promoting an immunosuppressive environment that benefits tumor survival. Targeting CCR8 presents a novel cancer immunotherapy strategy, aiming to dismantle this suppression without broader Treg depletion's adverse effects. Recent studies highlight that anti-CCR8 therapies, particularly monoclonal antibodies in clinical trials, may enhance anti-tumor immunity by reducing Treg-mediated suppression.

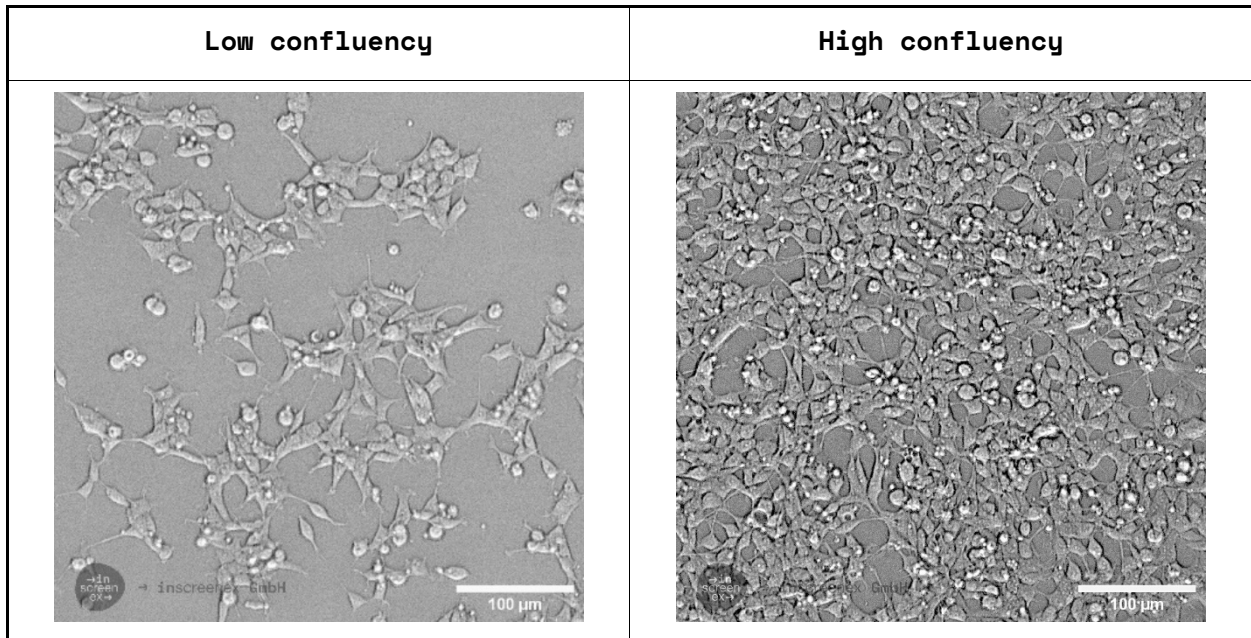
Note	<i>All target sequences undergo codon optimization and other sequence modifications on DNA level to improve recombinant expression and the nucleotide sequence of the recombinant protein therefore differs from database reference sequences. For details refer to section Target Sequence on page 11.</i>
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Cell Line Generation

This cell line was generated using our inscreenex 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

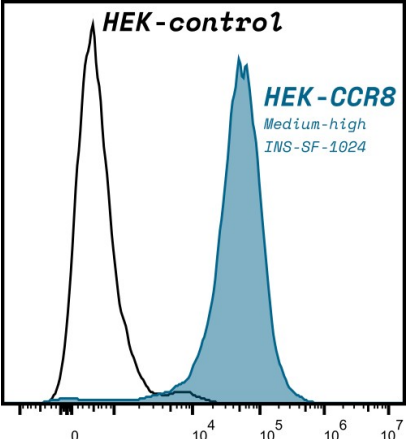


→ Cell Characterization

Target Expression Level

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

Materials	Protocol
<ul style="list-style-type: none"> – PBS/EDTA solution – 2% FBS/FCS in PBS (FACS Buffer) – Primary antibody: anti human CD198 (CCR8)-PE labelled, (Biolegend #360603) 	<p>Wash Protocol: Add FACS Buffer, resuspend cells gently, then centrifuge at 300×g for 5min.</p> <ol style="list-style-type: none"> 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×.

Expression Profile	
 <p>HEK-control: HEK293 landing pad cell line transfected with a scrambled sequence.</p> <p>HEK-CCR8: HEK293 landing pad cell line transfected with the target.</p> <p>→ Both samples were stained with antibodies and analyzed according to the Protocol section.</p>	<ol style="list-style-type: none"> 5) Add primary antibody in FACS buffer, resuspend cells gently. 6) Incubate at ambient temperature for 20-30min. 7) Wash cells 2x. 8) Resuspend in 100-200µl FACS Buffer. 9) Analyze cells using a flow cytometer.

Receptor Density

Receptor density was estimated using the BD Quantibrite™ System.

Materials	Protocol
<ul style="list-style-type: none"> – BD Quantibrite™ PE Phycoerythrin Fluorescence Quantitation Kit (#340495) – 2% FBS/FCS in PBS (FACS Buffer) 	<p>For a detailed protocol refer to the manufacturer's protocol (Link).</p> <ol style="list-style-type: none"> 1) Reconstitute Beads in FACS Buffer. 2) Measure Beads in the same run as cells using the same flow cytometer settings. 3) Convert Signal to PE molecules/cell according to manufacturer's instructions.
<p>Expression Level</p>	
<p>22000 molecules/cell</p>	

→ 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-CCR8 cell line and are either required or recommended for a successful cell culture.

Required	Recommended
Medium: HEK293 Growth Medium A (INS-ME-1041) Coating solution: Collagen Coating (INS-SU-1017) for best attachment after thawing.	Cryopreservation: Cell Freezing Medium (INS-SU-1027) Other expression levels: CHO-CCR8 Medium-high (INS-SF-1023)

→ 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:
<ul style="list-style-type: none">– 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	<ol style="list-style-type: none">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:	
<ul style="list-style-type: none">– 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	<p><i>For best attachment and viability after thawing the cells, we recommend using Collagen-coated flasks or plates for the initial seeding after cryo-recovery.</i></p> <p><i>Collagen coating is not required for subsequent routine culture of the cells.</i></p>
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Required materials	Protocol																
<ul style="list-style-type: none"> – Cell culture plastic (flasks, dishes, plates) – Sterile PBS – Collagen Coating (INS-SU-1017) 	<ol style="list-style-type: none"> 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																	
<ul style="list-style-type: none"> – 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																	
<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="padding: 2px 5px;"><i>Flask/Plate</i></th> <th style="padding: 2px 5px;"><i>Coating Volume</i></th> </tr> </thead> <tbody> <tr><td style="padding: 2px 5px;">T75</td><td style="padding: 2px 5px;">3ml</td></tr> <tr><td style="padding: 2px 5px;">T25</td><td style="padding: 2px 5px;">1ml</td></tr> <tr><td style="padding: 2px 5px;">6-well</td><td style="padding: 2px 5px;">0.7ml</td></tr> <tr><td style="padding: 2px 5px;">12-well</td><td style="padding: 2px 5px;">0.25ml</td></tr> <tr><td style="padding: 2px 5px;">24-well</td><td style="padding: 2px 5px;">0.1ml</td></tr> <tr><td style="padding: 2px 5px;">48-well</td><td style="padding: 2px 5px;">75µl</td></tr> <tr><td style="padding: 2px 5px;">96-well</td><td style="padding: 2px 5px;">50µl</td></tr> </tbody> </table>	<i>Flask/Plate</i>	<i>Coating Volume</i>	T75	3ml	T25	1ml	6-well	0.7ml	12-well	0.25ml	24-well	0.1ml	48-well	75µl	96-well	50µl	
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→ Medium Information

Note	<i>We provide a ready-to-use HEK293 Growth Medium A (INS-ME-1041) and Cell Freezing Medium (INS-SU-1027) for the culture and cryopreservation of stable HEK293 cells.</i>
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Ready-to-use HEK293 Growth Medium A

Selection antibiotic	Anti-contamination antibiotics
<i>Our HEK293 Growth Medium A is shipped ready to use and already contains the G418 selection antibiotic (1mg/ml final) to guarantee stable long-term expression of the target.</i>	<i>Our HEK293 Growth Medium A 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.</i>

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

Stability: See Expiry Date on bottle label.

→ 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	<i>For best attachment and viability after thawing the cells, we recommend using Collagen-coated flasks or plates for the initial seeding after cryo-recovery.</i> <i>Collagen coating is not required for subsequent routine culture of the cells.</i>
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Required materials	Protocol
<ul style="list-style-type: none"> – Coated cell culture vessel (see section Coating Protocol on page 6) – HEK293 Growth Medium A (INS-ME-1041) pre-warmed to 37°C – 15ml tube with a conical bottom suitable for centrifugation (e.g. "Falcon tube") 	<ol style="list-style-type: none"> 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
<ul style="list-style-type: none"> – 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") 	<ol style="list-style-type: none"> 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% CO₂ (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			Protocol
<ul style="list-style-type: none"> – HEK293 Growth Medium A (INS-ME-1041) – 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	

- 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.

