



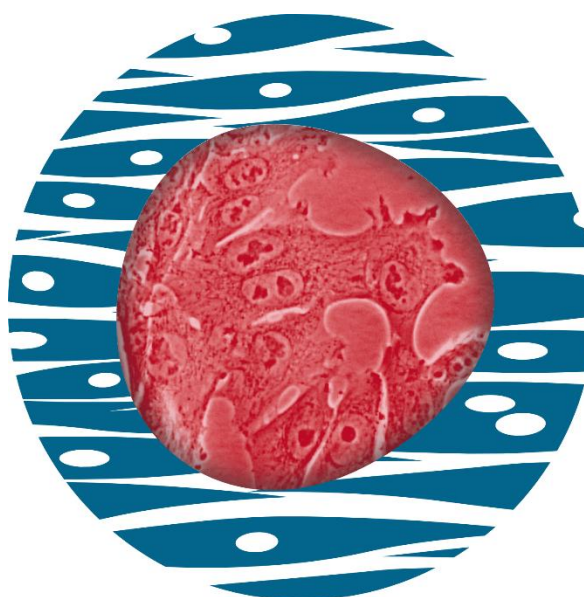
CHO-NECT4

Cat.-No.: INS-SF-1057

NECT4 Expressing Stable Recombinant CHO Cell Line

Product Sheet

→ At a glance		
BSL Level 1	Coating not required	Growth Adherent, Suspension
Storage <2d: -80°C >2d: liquid Nitrogen	Medium CHO Growth Medium D (INS- ME-1048)	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

CHO-NECT4 is a recombinant CHO cell line expressing full length human NECT4 (Nectin-4).

Catalog number: INS-SF-1057

Target: human NECT4

Target aliases: Ig superfamily receptor LNIR, Poliovirus receptor-related protein 4

Target expression level(s): n. d.

Biosafety level (BSL): Level 1

Cell background: CHO (*Cricetulus griseus*; Chinese hamster)

Growth properties: adherent, can be transferred to suspension

Target Background

Nectin-4 is a single-pass type I membrane protein of the nectin family of immunoglobulin-like adhesion molecules. It contains two Ig-like C2-type domains and one Ig-like V-type domain. The protein mediates Ca²⁺-independent cell-cell adhesion through trans-homophilic and heterophilic interactions, including interactions with NECTIN1, and is a key component of adherens junctions. Nectins bind the actin cytoskeleton through the adaptor protein afadin. A soluble form is generated by proteolytic cleavage at the cell surface by ADAM17/TACE and is detected in breast tumor cell lines and patients. Nectin-4 does not act as a receptor for alpha-herpesvirus entry but serves as a receptor for measles virus. Mutations cause ectodermal dysplasia-syndactyly syndrome type 1. Alternatively spliced transcript variants have been described.

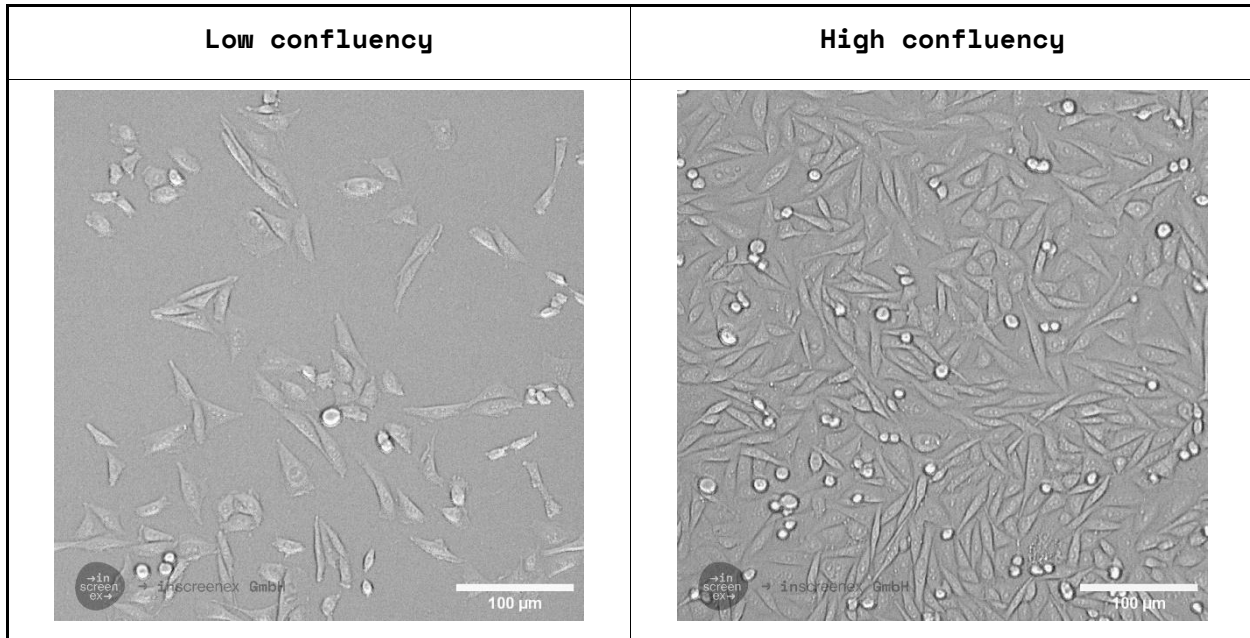
Note	<p><i>All target sequences undergo codon optimization and other sequence modifications on DNA level to improve recombinant expression and the nucleotide sequence of the recombinant protein therefore differs from database reference sequences. For details refer to section Target Sequence on page 8.</i></p>
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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.

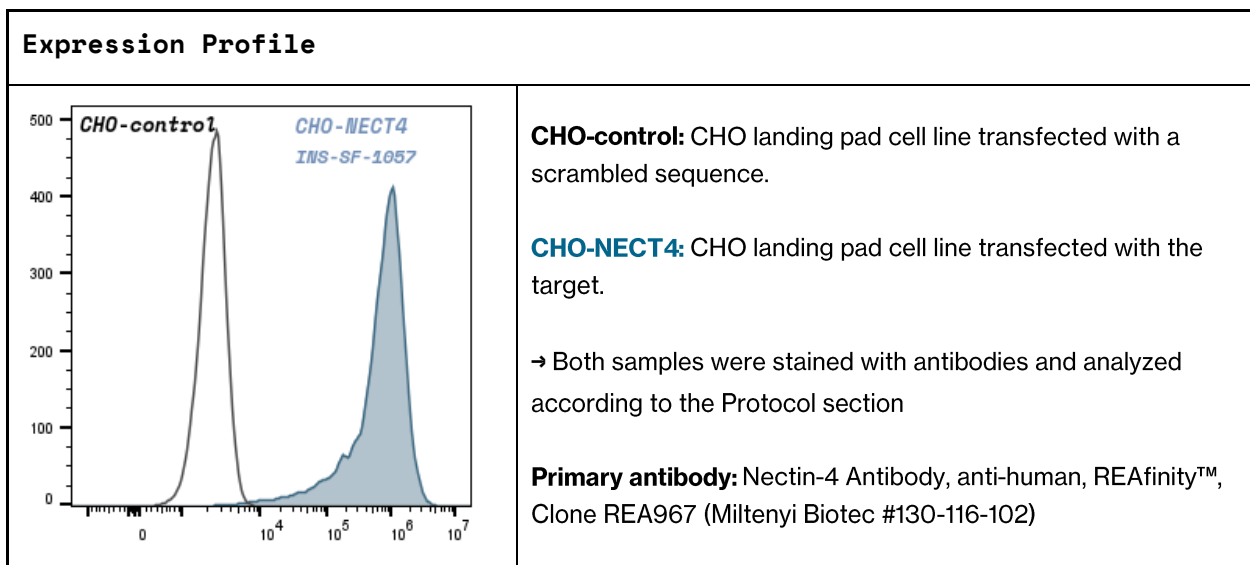
→ Morphology

adherent; epithelial-like; grows in monolayer



→ Cell Characterization

Target expression 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: Nectin-4 Antibody, anti-human, REAfinity™, Clone REA967 (Miltenyi Biotec #130-116-102) 	<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×. 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

Required or recommended products or consumables related to this cell line.

Required	Recommended
<p>Medium: CHO Growth Medium D (INS-ME-1048).</p> <p>Coating solution: not required</p>	<p>Cryopreservation: Cell Freezing Medium (INS-SU-1027)</p>



→ 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.	

→ Medium Information

Note	<i>We provide a ready-to-use CHO Growth Medium D (INS-ME-1048) and Cell Freezing Medium (INS-SU-1027) for the culture and cryopreservation of stable CHO-NECT4 cells.</i>
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Storage: Store CHO Growth Medium D 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 CHO Growth Medium D is shipped ready to use and already contains the selection antibiotic, Blasticidin (6µg/ml), to guarantee stable long-term expression of the target.	Our CHO Growth Medium D 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 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.

Required materials	Protocol
<ul style="list-style-type: none"> – Cell culture vessel – CHO Growth Medium D (INS-ME-1048) 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 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. Cells may also be frozen directly from suspension culture.

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 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"> – CHO Growth Medium D (INS-ME-1048) – PBS – Trypsin/EDTA solution (TE) 			
Recommended volumes			
Flask or Plate	Medium or PBS	TE solution	<ol style="list-style-type: none"> 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.
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	

→ Target Sequence

Note	<p>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.</p>
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Amino Acid Sequence (Protein)

Uniprot ID: Q96NY8

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MPLSLGAEMWGPFAWILLLLLLASFTGRCPAGELETSDVVTVVLGQDAKLPFCFYRGDSGEQVQVAVARVDAGEGAQELALLHSKYGLHVSPAYEGRVEQPPPPRNPLDGSVLLRNAVQADEGEYECRVSTFPAGSFQARLRLRVLVPLPSL
NPGPALEEGGQLTLAASCTAEGSPAPSVTWDTEVKGTTSSRSFKHSRGAAVTSEFHLVPSRSMNGQPLTCVVSHPGLLQDQRITHILHVSFLAEASVRGLEQNLWHIGREGAMLKCLSEGQPPPSYNNWTRLDGPLPSGVRVDGDTLGFPPPLT
TEHSGIYVCHVSNFSSRDSQVTVDLVDPQEDSGKQVDLVSASVVVGVIAALLFCLLVVVVLMRSYHRRKAQMTQKYEEELTLTRENISIRRLHSHHTDPRSQPEESVGLRAEGHPDSLKDNSSCSVMSEEPGRSYSTLTTVREIETQTE
LLSPGSGRAEEEDQDEGIKQAMNHVQENGLRAKPTGNGIYINGRGLV
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