



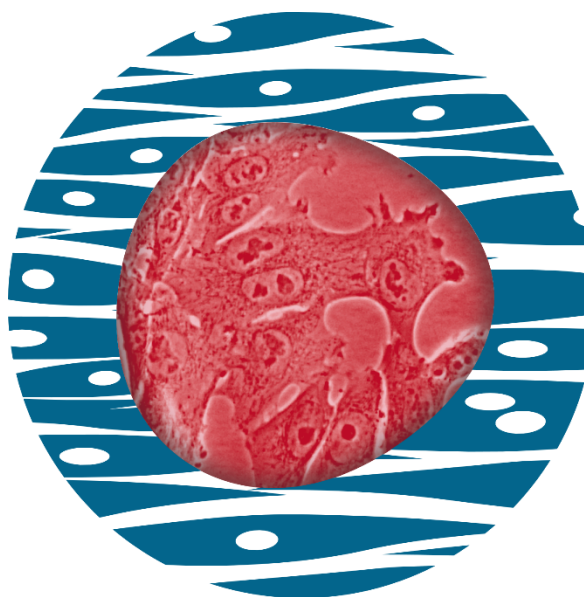
CHO-B7H3

Cat.-No.: INS-SF-1019

B7H3 Expressing Stable Recombinant CHO Cell Line

Product Sheet

→ At a glance		
BSL <i>Level 1</i>	Coating <i>not required</i>	Growth <i>Adherent, Suspension</i>
Storage <i><2d: -80°C >2d: liquid Nitrogen</i>	Medium <i>CHO Growth Medium A (INS- ME-1039)</i>	Expression Level <i>High (430000 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
→ Medium Information	7
Ready-to-use CHO Growth Medium A	7
Suspension Culture Medium	7
→ Thaw Cryopreserved Cells	8
→ Freeze Cells for Cryopreservation	8
→ Routine Adherent Cell Culture	9
→ Suspension Culture	10
→ Target Sequence	11
Amino Acid Sequence (Protein)	11
Nucleotide sequence (DNA)	11
→ References	11

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→ Intended Use and Licensing

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→ Background Information

CHO-B7H3 is a recombinant CHO cell line expressing full length human B7H3.

Catalog number: INS-SF-1019

Target: human B7H3

Target aliases: CD276, B7-H3; B7H3, B7RP-2, B7 Homolog 3, 4Ig-B7-H3

Target expression level(s): High (approx. 430000 molecules/cell)

Biosafety level (BSL): Level 1

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

Growth properties: adherent, can be transferred to suspension

Target Background

B7-H3 (also known as CD276) belongs to the B7 family, regulating immune response by binding to CD28 receptors on lymphocytes. It is overexpressed in many cancers, acting as a negative regulator of T- cell immune responses, linked to poor patient outcomes. B7-H3 is implicated in tumor progression, including angiogenesis, metastasis, and exosomal activity. The soluble form (sB7-H3) can be released into the tumor microenvironment, which is associated with increased migration and invasion of cancer cells. Due to its selective presence in tumor tissues and multifaceted effects on cancer cells, B7-H3 is considered an attractive target for immunotherapy.

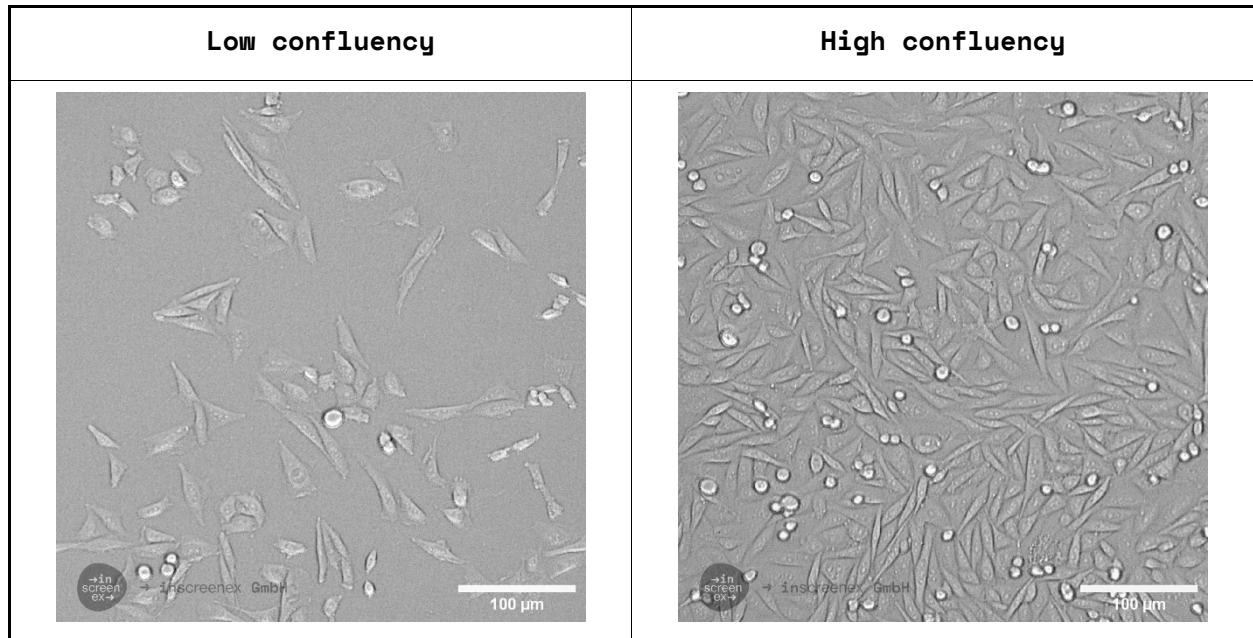
Note	<i>All target sequences undergo codon optimization and other sequence modifications on DNA level to improve recombinant expression and the nucleotide sequence of the recombinant protein therefore differs from database reference sequences. For details refer to section Target Sequence on page 10.</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

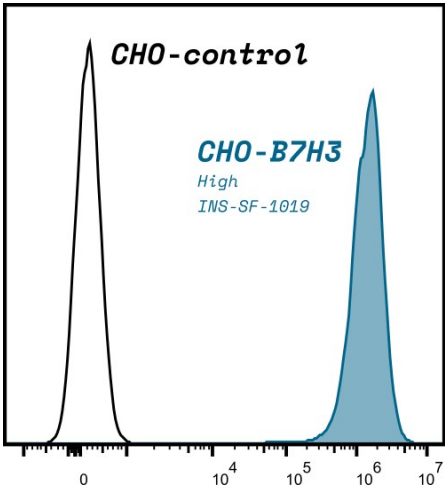


→ Cell Characterization

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 CD276-PE labelled, (Miltenyi Biotech, #130-118-570) 	<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.

Expression Profile	
 <p>CHO-control: CHO landing pad cell line transfected with a scrambled sequence.</p> <p>CHO-B7H3: CHO 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"> 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>430000 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 CHO-B7H3 cell line and are either required or recommended for a successful cell culture.

Required	Recommended
Medium: CHO Growth Medium A (INS-ME-1039). Coating solution: not required	Cryopreservation: Cell Freezing Medium (INS-SU-1027) Other expression levels: not available

→ 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 A (INS-ME-1039) and Cell Freezing Medium (INS-SU-1027) for the culture and cryopreservation of stable CHO-B7H3 cells.</i>
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Ready-to-use CHO Growth Medium A

Selection antibiotic	Anti-contamination antibiotics
Our CHO 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.	Our CHO 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.

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

Stability: See Expiry Date on bottle label.

Suspension Culture Medium

We have tested the medium formulation indicated below for successful suspension culture of CHO-B7H3 cells.

Note	<i>While we have only tested the medium formulation indicated below, the cells can be adapted to grow in most of the commercially available CHO suspension media (e.g. Lonza ProCHO 5 #BELN12-766Q). Just make sure to follow standard CHO suspension culture adaption protocols.</i>
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Components	Final concentration	Volumes for 1000ml basal medium
Suspension Medium		
CD Hybridoma Medium Gibco #11279023	n.a.	1000ml
L-Glutamine (200mM) e.g. Gibco #25030081	8mM	40ml
Cholesterol Lipid Concentrate (250x) e.g. Gibco #12531018	1x	4ml
Geneticin aka G418-sulfat (50mg/ml) e.g. Gibco™ #10131035	0.5mg/ml	10ml
[Optional] Anti Clumping Agent* e.g. Gibco #0010057AE	use at manufacturer's recommended concentration	varying

*Can be used to reduce cell aggregation



→ 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 A (INS-ME-1039) 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.
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→ 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"> – CHO Growth Medium A (INS-ME-1039) – 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.



→ Suspension Culture

The CHO-B7H3 cell line can be transferred to suspension culture starting from standard adherent culture. Work in a sterile environment and follow Good Cell and Tissue Culture Practice.

Temperature: 37°C

Environment: 5% CO₂ (v/v), humidified atmosphere

Seeding density: 2-4×10⁵ cells/ml

Split ratio: adjust to 2-4×10⁵ cells/ml every third day

Medium change: feed cells with fresh medium to seeding density if medium appears used

Shaking: 110rpm at Ø 25mm throw

Required materials	Protocol
<ul style="list-style-type: none">– Suspension Medium See section Suspension Culture Medium on page 7– Adherent cell culture for initial seeding– PBS– 5% FBS (v/v) in PBS– Trypsin/EDTA solution (TE)– 15ml tube with a conical bottom suitable for centrifugation (e.g. "Falcon tube")– Erlenmayer flask 250ml e.g. Corning #431144– Shaker e.g. Infors Celltron	<ol style="list-style-type: none">1) Aspirate medium from adherent culture.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 5% FBS in PBS thereby inactivating the Trypsin/EDTA (TE) solution.6) Centrifuge cells at 300×g for 5min.7) Aspirate supernatant.8) Resuspend cell pellet in 5ml Suspension Medium.9) Adjust cell density to 2-4×10⁵ cells/ml in 50ml Suspension Medium.10) Transfer cell suspension to Erlenmayer flask for shaking in incubator.



→ Target Sequence

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

Uniprot ID: Q5ZPR3

<pre>>sp Q5ZPR3 CD276_HUMAN CD276 antigen OS=Homo sapiens OX=9606 GN=CD276 PE=1 SV=1 MLRRRSGSPGMGVHVGALGALWFLTLGALVQVPEDPVVALVGTDLTLCFSFSEPFGLS AQLNLIWQLTDTKQLVHSFAEQDQGSAYANRTALFPDLLAQGNASRLRQRVVADEGSF TCFVSIRDGSAAVSLQVAAPYSKPSMTLEPNKDLRPGDVTITCSSYQGYPEAEVFWQD GGQVPLTGNVTTSQMANEQGLFDVHSILRVVLGANGTYSCLVRNPVLQDDAHSSVITIPQ RSPTGAVEVQVPEDPVVALVGTDLTLCFSFSEPFGLS AQLNLIWQLTDTKQLVHSFTEG RDQGSAYANRTALFPDLLAQGNASRLRQRVVADEGSFTCFVSIRDGSAAVSLQVAAPY SKPSMTLEPNKDLRPGDVTITCSSYRQGYPEAEVFWQDGGVPLTGNVTTSQMANEQGLF DVHSVLRVVLGANGTYSCLVRNPVLQDDAHGVSITIGQPMTFPEALWTVGLSVLCLIAL LVALAFVCRKIKQSCSEENAGAEDQDGEGETALQPLKHSKEDDQGEIA</pre>
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Nucleotide sequence (DNA)

<pre>atgctgaggagaagagcctctcctggaatgggagtgcatgtggagctgctctgggagccctgtggttttgctgactggcgcctggaagtgcaggtccagagagatcctgtggtggcccttgggaaacagatgccacactgtgctg cagcttaagccctgagcctggattttctctggcccagctgaacctgatctggcagctgaccgatccaagaagctggtgacacagctttgccaaagccaggatcaggaagcgcctacgccaatagaaccgctctgtccccgatctgc tgcccagggaaatgacctctctgagactgacagagagtgccgggttgccgacgagggcagcttcaactgttctggtccatcagagacttcggcagcgcctgtgtctctgcaagtggcgcctcttacagcaagcccagcatgacctg gaacctaaccaagcctgagcctggcgacacogtgaccatacaatgtagcagctaacagggtcaccctgaggccgaagtgtttggcagagatggacagggcgtgccctgaccggaaatgtgcaacaacagccagatggccaacagca ggccctgttctgctgcaacagatcctgagagtggtgctgggcccacatggcaacctctctgtctgtgccaatccctgctcgcagcagagtgcccaacagcagcgtgacaatcaacctcaagatctccaacagggcgcctggaag tgaagtgcctgaagatccagtggtgctctctgtgggaccgacgctacactgagatgtagctttagcccagcagcaggttcagcctggctcagctcaatctcaattggcagctcacagacacaaaacagctggtccactccttacc gaagggcagagatcagggatctgctatgccaaccggacagccctgttctctgatctctctgctcaaggcaatgccagcctgaggtccagagagtcagagtcctgacagggcctcctttacatgctctgtcagcatccgagactcagg ctctgtctgagttagctgcaggtcgcaagcccttactccaagcctccatgacacttgagcccaacaagatctcagaccggcgatcacagtgaccattacctgagctcctacagagctaccccgaagctgaggtttctggcaag acggccaaggggtgccactgacagggcaacgtaaccacatcagatggttaatgaacagggactcttgactgcaactcctgctgaggggtgtgctgggagctaacggcacatcacagctgacctgtgaggaaacctgtgctcagca gacgccatgctcctgtaacaattaccggccagcctatgaccttccaccagaggtctgtgggtcaactggcctgtctgtgtgtctgattgacctgctggtgctctggccttgggtgtgctggcgaagatcaagcagagctgca ggaaagaaacgctggcgcgaagatcaggtggcgaagggcaggggaagcaagacagctctgcagcctctgaagcacagcagacgaagagcagcagggacagcagcagagatcgcc</pre>
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→ References

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