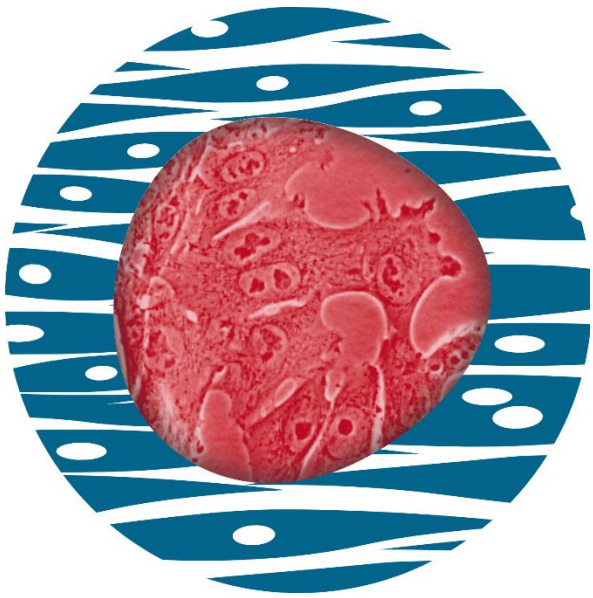
CHO-IL2Rα

Cat.-No.: INS-SF-1045

IL2Ra 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*** *High* *175600 molecules/cell*



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→ Intended Use and Licensing

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→ Background Information

CHO-IL2Ra is a recombinant CHO cell line expressing full length human IL2Ra (Interleukin 2 Receptor Subunit Alpha).

Catalog number: INS-SF-1045

Target: human IL2Ra

Target aliases: CD25, TAC-Antigen

Target expression level(s): High (175600 molecules/cell)

Biosafety level (BSL): Level 1

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

Growth properties: adherent, can be transferred to suspension

Target Background

Interleukin 2 receptor subunit alpha (IL2RA) is a cell surface receptor subunit for interleukin-2. Together with IL2RB and IL2RG it constitutes the high-affinity IL2 receptor; homodimeric IL2RA forms a low-affinity receptor, and homodimeric IL2RB forms a medium-affinity receptor. IL2RA contributes to IL2 binding affinity but not to recruitment of signaling molecules. It is constitutively expressed on regulatory T cells and is involved in tolerance regulation and T-cell expansion, contributing to immune tolerance by controlling regulatory T cell activity that suppresses activation and expansion of autoreactive T cells. Soluble IL2RA can arise from extracellular proteolysis. Alternative splicing yields multiple transcript variants. Mutations are associated with interleukin 2 receptor alpha deficiency.

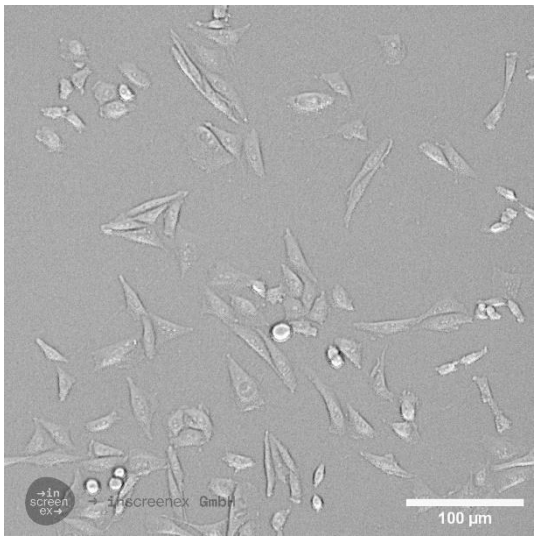
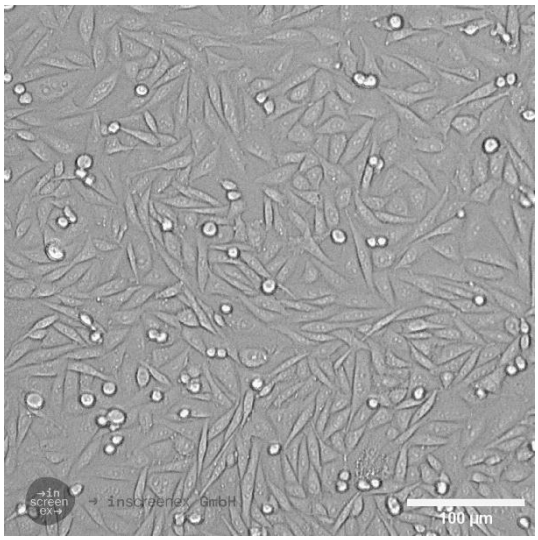
Note	*All target sequences undergo codon optimization and other sequence modifications on DNA level to improve recombinant expression and the nucleotide sequence of the recombinant protein therefore differs from database reference sequences. For details refer to section* *Target Sequence on page 8**.*

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

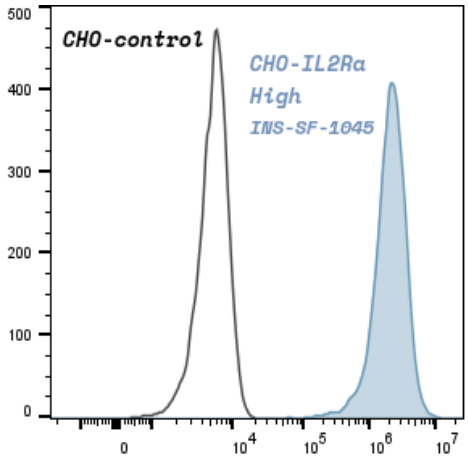
adherent; epithelial-like; grows in monolayer

Low confluency	High confluency
	

→ Cell Characterization

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

Expression Profile



CHO-control: CHO landing pad cell line transfected with a scrambled sequence.

CHO-IL2Ra: CHO landing pad cell line transfected with the target.

→ Both samples were stained with antibodies and analyzed according to the Protocol section

Primary antibody: PE anti-human CD25 Antibody, Clone M-A251 (BioLegend #356103)

Materials	Protocol
– PBS/EDTA solution – 2% FBS/FCS in PBS (FACS Buffer) – Primary antibody: PE anti-human CD25 Antibody, Clone M-A251 (BioLegend #356103)	**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.

Receptor Density	
175600 molecules/cell	**Materials** – BD Quantibrite™ PE Phycoerythrin Fluorescence Quantitation Kit (#340495) – 2% FBS/FCS in PBS (FACS Buffer) **Protocol** For a detailed protocol refer to the manufacturer's protocol (Link). 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.

→ 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
Medium: CHO Growth Medium D (INS-ME-1048). **Coating solution:** not required	**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.	

→ Medium Information

Note	*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-IL2Ra cells.*

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
– 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")	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
– 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 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

- CHO Growth Medium D (INS-ME-1048)
- 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: *P01589*

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MDSYLLMWGLLTFIMVPGCQAELCDDDPPEIPHATFKAMAYKEGTMLNCECKRGFRRIKSGSLYMLCTGNSSHSSWDNQCQCTSSATRNTTKQVTPQPEEQKERKTTEMQSPMQPVDQASLPGHCREPPPWENEATERIYHFVVGQMVYYQCV
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Nucleotide sequence (DNA)

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→ References

We would love to hear about your research! Please let us know if you have published using our cells.