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Data Sheet

CD33– CHO K1 Recombinant Cell Line (Medium Expression)

Catalog # 79935-M

Background

CD33 or Siglec-3 is a transmembrane receptor expressed on cells of myeloid lineage that belongs to the sialic acid-binding immunoglobulin (Ig)-like family. CD33 can be stimulated by sialic acid residues on glycoproteins or glycolipids, resulting in a signaling cascade that inhibits phagocytosis in cells and suppresses immune cell proliferation. CD33 overexpression is one of top-ranked risk factors for Alzheimer's disease, and CD33 is the target of Gemtuzumab ozogamicin, an antibody- chemo drug conjugate for the treatment of patients with acute myeloid leukemia.

Description

Recombinant CD33-CHO K1 cell line stably expressing the full length human C33/Siglec-3 receptor (GenBank Accession: BC028152.1). Surface expression of CD33 was confirmed by flow cytometry. Each stable clonal cell line was selected for medium level of CD33 expression to mimic different stages of cancer target cells with medium CD33 expression levels.

Application

- Screen for activators or inhibitors of antibody-mediated signaling for immunotherapy research and drug discovery.
- Characterize CD33 antibodies and ligands for binding assay.

Format

Each vial contains ~ 2 x 10⁶ cells in 1 ml of 10% DMSO in FBS.

Storage

Store in liquid nitrogen immediately upon receipt.

Culture Medium

Thaw Medium 3: (BPS Bioscience #60186): Ham's F-12 medium (Hyclone #SH30526.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

Growth Medium 3B (BPS Bioscience #79529): Thaw Medium 3 (BPS Bioscience #60186) plus 500 µg/ml Hygromycin B (Invitrogen #10687010)

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Recommended Culture conditions

Frozen Cells: Prepare T-75 culture flask with 20 ml of pre-warmed Thaw medium 3. Quickly thaw cells in a 37°C water bath with constant and slow agitation. After cleaning the outside of the vial with 70% ethanol, immediately transfer the entire content to Thaw Medium 3 (no Hygromycin). Avoid pipetting up and down, and gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO₂. The next day, change to fresh Thaw Medium 3 without disturbing the attached cells. Continue to incubate until cells reach desired confluency. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture.

Subculture: When cells reach 90% confluency, remove the medium and wash twice with PBS (without Magnesium or Calcium). Treat cells with 2 ml of 0.25% trypsin/EDTA and incubate for 3 minutes at 37°C. After confirming cell detachment by light microscopy, add 10 ml of prewarmed medium and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes. Remove the medium and resuspend cells in 10 ml pre-warmed growth medium. Dispense 1 ml of the cell suspension into a new T75 flask containing pre-warmed 19 ml complete medium (a subcultivation ratio of 1:10 to 1:20 is recommended). Incubate cells in a humidified 37°C incubator with 5% CO₂. To freeze cells, resuspend cell pellet in freezing medium (10% DMSO in FBS).

Mycoplasma Testing

This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, #LT07-518) was used as a positive control.

Validation

Cell surface expression of human CD33 in CHO K1 cells was confirmed by flow cytometry.

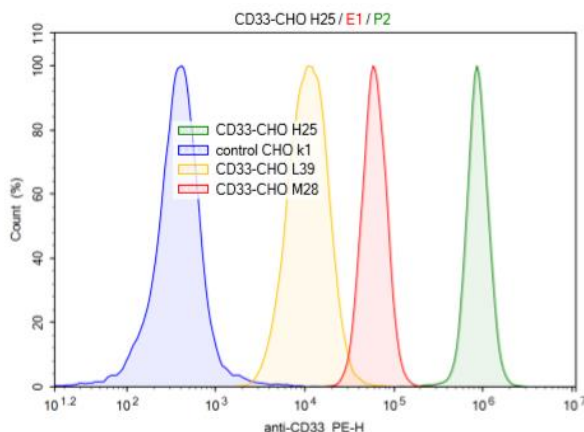
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Figure 1. Flow cytometry analysis of cell surface expression of CD33 in CHO K1 cells. CD33-CHO K1 cells or control CHO K1 cells were stained with PE-labeled anti-human CD33 antibody (Biolegend #366607) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE.



Vector and Sequence

Human CD33/Siglec-3 (accession number: BC028152.1) was cloned into pCMV3 vector.

```
MPLLLLLLPLLWAGALAMDPNFWLQVQESVTVQEGLCVLPCTFFHPIPYDKNSPVHGYWVFREGAIIISGD
SPVATNKLDQEVQEETQGRFRLLDGPSRNNCSLSIVDARRRDNNGSYFFRMERGSTKYSPQLSVHVT
DLTHRPKILIPGTLEPGHSKNLTCSVSWACEQGTPIFSWLSAAPTSLGPRTTTHSSVLIITPRPQDHGTNLT
CQVKFAGAGVTTERTIQLNVTYVPQNPTTGIFPGDGSQKQETRAGVVHGAIGGAGVTALLALCLCLIFFIV
KTHRRKAARTAVGRNDTHPTTGSASPKHQKSKLHGPTETSSCSGAAPT VEMDEELHYASLNFGMNP
SKDTSTEYSEVRTQ
```

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| <u>Related Product</u> | <u>Cat. #</u> | <u>Size</u> |
|----------------------------------------------------|----------------------|--------------------|
| Thaw Medium 3 | 60186 | 500ml |
| Growth Medium 3B | 79529 | 500ml |
| CD33-CHO Recombinant Cell Line (Medium Expression) | 79935-M | 2 vials |
| CD33-CHO Recombinant Cell Line (Low Expression) | 79935-L | 2 vials |
| CD33-CHO Recombinant Cell Line (High Expression) | 79935-H | 2 vials |
| CD22-CHO Recombinant Cell Line (Medium Expression) | 79557-M | 2 vials |
| CD22-CHO Recombinant Cell Line (Low Expression) | 79557-L | 2 vials |
| CD22-CHO Recombinant Cell Line (High Expression) | 79557-H | 2 vials |

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