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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Anti-CD22 CAR-T Cells are produced by high-titer lentiviral transduction of human primary $CD4^+$and $CD8^+$ T cells using the Anti-CD22 CAR Lentivirus (Clone m971 ScFv-CD8-4-1BB-CD3ζ) (#78608). These ready-to-use CAR-T cells express an anti-CD22 CAR consisting of the ScFv (Single chain fragment variable) of anti-CD22(clone m971) linked to a 2nd generation CAR (Chimeric Antigen Receptor) containing CD8 hinge and transmembrane domains, and the 4-1BB and CD3ζ signaling domains (Figure 1).

These CAR-T cells have been validated using flow cytometry (to determine the CAR expression) and co-culture cytotoxicity assays.

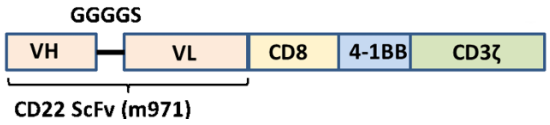

Figure 1. Construct diagram showing components of the anti-CD22 CAR expressed in Anti-CD22 CAR-T Cells.

Background

CD22, also known as Siglec-2, is a nearly universally expressed B cell surface antigen. It is reported to act as an inhibitory co-receptor of the B cell receptor to control the body's B cell response. In 2017 the FDA approved inotuzumab ozogamicin (Besponsa), an antibody-drug conjugate targeting CD22, for patients with B cell acute lymphoblastic leukemia (ALL). Outcomes are poor for patients with large B-cell lymphoma who relapse after CD19-targeted therapy. However, CD22 CAR-T cells have demonstrated high efficacy in pediatric and adult B-ALL in clinical trials, providing a therapeutic option for patients with $CD22^+$ malignancies who show disease progression after CD19-directed chimeric antigen receptor (CAR) T cell therapy.

Application

- Use as positive control in the development of anti-CD22 CAR-T cells.
- Screen modulators of anti-CD22 CAR-T cytotoxicity.
- Design and optimize co-culture cytotoxicity assays for anti-CD22 specific CAR-T cell evaluation.

Biosafety

Anti-CD22 CAR-T Cells are produced with the third generation SIN (self-inactivation) lenti-vector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle.

Materials Provided

Components	Format
One vial of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of CryoStor® CS10 (Stemcell Technologies #100-1061)

Mycoplasma Testing

The cells have been screened to confirm the absence of Mycoplasma species.

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Materials Required but Not Supplied

These materials are not supplied with the CAR-T cells but are necessary for cell culture and for the cellular assays described below. BPS Bioscience's reagents are validated and optimized for use with these cells and are highly recommended for best results.

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Thaw Medium 3	BPS Bioscience #60186
TCellM™	BPS Bioscience #78753
Human Interleukin-2 Recombinant	BPS Bioscience #90184
CD22, Fc Fusion, Avi-Tag, PE-labeled Recombinant	BPS Bioscience #101028
CD22 / Luciferase - CHO Recombinant Cell Line	BPS Bioscience #79715
Firefly Luciferase - CHO Recombinant Cell Line	BPS Bioscience #79725
Firefly Luciferase Raji Cell Line	BPS Bioscience #78622
Firefly Luciferase K562 Cell Line	BPS Bioscience #78621
Untransduced T Cells (Negative Control for CAR-T cells)	BPS Bioscience#78170
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

Recommended Anti-CD22 CAR-T Cell Medium: TCellM™ (#78753) supplemented with 10 ng/ml Interleukin-2 (#90184).

Cell Culture Protocol

Cell thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Anti-CD22 CAR-T Cell Medium.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Anti-CD22 CAR-T Cell Medium.

3. Transfer the resuspended cells to a T25 flask.

Cell culture

1. Centrifuge the cells gently at 300 *x g* for 5 minutes.

2. Resuspend in fresh Anti-CD22 CAR-T Cell Medium.

3. Continue to culture the cells at 37°C with 5% CO_2.

4. Do not allow the cell density to exceed 2.0 x 10^6 cells/ml. Transfer the cells in larger culture vessels and add fresh medium when the density reaches 2.0 x 10^6 cells/ml.

Validation Data

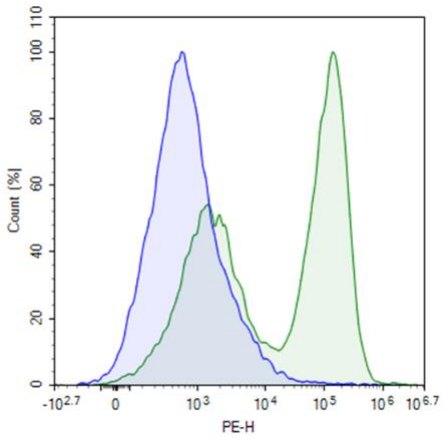

Figure 2. Expression of anti-CD22 CAR in anti-CD22 CAR-T cells.
Anti-CD22 CAR-T cells were thawed for 24 hours and stained with CD22, Fc Fusion, Avi-Tag, PE-labeled Recombinant (101028). Anti-CD22 expression was analyzed by flow cytometry. The y axis represents the % of cells, while the x axis indicates PE-intensity. Green: Anti-CD22 CAR-T Cells; Blue: Untransduced T cells.

Functional Validation

<u>Cytotoxicity assay using Firefly Luciferase Raji Cell Line as the target cells.</u>

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include "Luminescence Background", "No T Cell Control" (contain Firefly Luciferase Raji cells but no T cells) and "Test Condition" wells.
- The following experiment is an example of co-culture assay used to evaluate the cytotoxicity of Anti-CD22 CAR-T Cells against Firefly Luciferase Raji Cell Line as the target cells.
- We recommend using Untransduced T Cells (as negative control for anti-CD22 CAR-T cells).
- We recommend using Firefly Luciferase K562 Cell Line as negative control.

Day 1:

1. Thaw T cells, activate if desired, and expand according to the protocol in the "**Cell Culture Protocol"** section.

2. Seed Firefly Luciferase Raji cells, which express endogenous CD22, at 5,000 cells/well in 50 µl of Thaw Medium 2 in a 96-well white, clear bottom tissue culture plate. Leave a few empty wells as "Luminescence Background" wells.
3. Centrifuge T cells at 300 *x g* for 5 min and resuspended the cell pellet in fresh Anti-CD22 CAR-T Cell Medium.

4. Determine the desired Effector to Target ratio (E:T) and prepare appropriate cell suspensions (50 µl/well).

5. Carefully pipet 50 µl of T cell suspension into the appropriate "Test Condition" wells, containing the Firefly Luciferase Raji cells.

6. Add 50 µl of Anti-CD22 CAR-T Cell Medium to the "No T Cell Control" wells.

7. Add 100 µl of Anti-CD22 CAR-T Cell Medium to the "Luminescence Background" wells.

8. Incubate the plates at 37°C with 5% CO_2 for 24 hours.

Day 2:

1. Add 100 µl of ONE-Step™ Luciferase assay reagent to each well.

2. Incubate at room temperature (RT) for ~15 to 30 minutes.

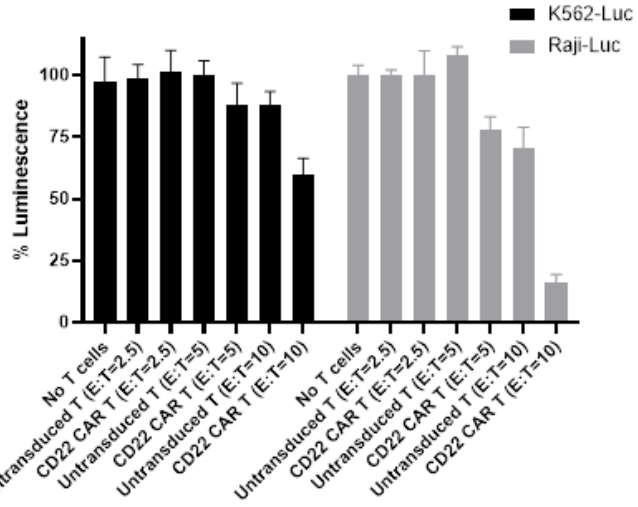

Figure 3. Luciferase-based cytotoxicity assay using Firefly Luciferase Raji Cell Line as the target cells.
Anti-CD22 CAR-T cells and control untransduced T cells (#78170) were thawed for 24 hours. The Anti-CD22 CAR-T cells (effector) were then co-cultured with Firefly Luciferase Raji Cells (target) for 24 hours at the indicated effector:target (E:T) ratio. The lysis of target cells was determined by measuring luciferase activity. Untransduced T cells (#78170) and Firefly Luciferase K562 Cell Line (#78621) were used as negative controls.

Cytotoxicity assay using CD22 / Luciferase - CHO Recombinant Cell Line as the target cells.

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include "Luminescence Background", "No T Cell Control" (contain CD22/Firefly Luciferase CHO cells but no T cells) and "Test Condition" wells.
- The following experiment is an example of co-culture assay used to evaluate the cytotoxicity of anti-CD22 CAR-T Cells against CD22 / Luciferase - CHO Recombinant Cell Line as the target cells.
- We recommend using Untransduced T Cells (as negative control for Anti-CD22 CAR-T Cells).
- We recommend using Firefly Luciferase- CHO Recombinant Cell Line as negative control.

Day 1:

1. Thaw T cells, activate if desired, and expand according to the protocol in the "**Cell Culture Protocol"** section.

2. Seed CD22 / Luciferase - CHO cells at 500 cells/well in 50 µl of Thaw Medium 3 in a 96-well white, clear bottom tissue culture plate. Leave a few empty wells as "Luminescence Background" wells.

3. Centrifuge T cells at 300 *x g* for 5 min and resuspended the cell pellet in fresh Anti-CD22 CAR-T Cell Medium.

4. Determine the desired Effector to Target ratio (E:T) and prepare appropriate cell suspensions (50 µl/ well).

5. Carefully pipet 50 µl of T cell suspension into the appropriate "Test Condition" wells, containing CD22 / Luciferase - CHO cells.

6. Add 50 µl of Anti-CD22 CAR-T Cell Medium to the "No T Cell Control" wells.

7. Add 100 µl of Anti-CD22 CAR-T Cell Medium to the "Luminescence Background" wells.

8. Incubate the plates at 37°C with 5% CO_2 for 24 hours.

Day 2:

1. Add 100 µl of ONE-Step™ Luciferase assay reagent to each well.

2. Incubate at RT for ~15 to 30 minutes.

Data Analysis: the average background luminescence was subtracted from the luminescence reading of all wells. The luciferase activity of Firefly Luciferase Cell Line ("No T Cell Control"- "Luminescence Background") was set as 100%. The % Luminescence was calculated as: (luminescence of "Test

Condition"- luminescence of "Luminescence Background")/ (luminescence of "No T Cell Control"- luminescence of "Luminescence Background").

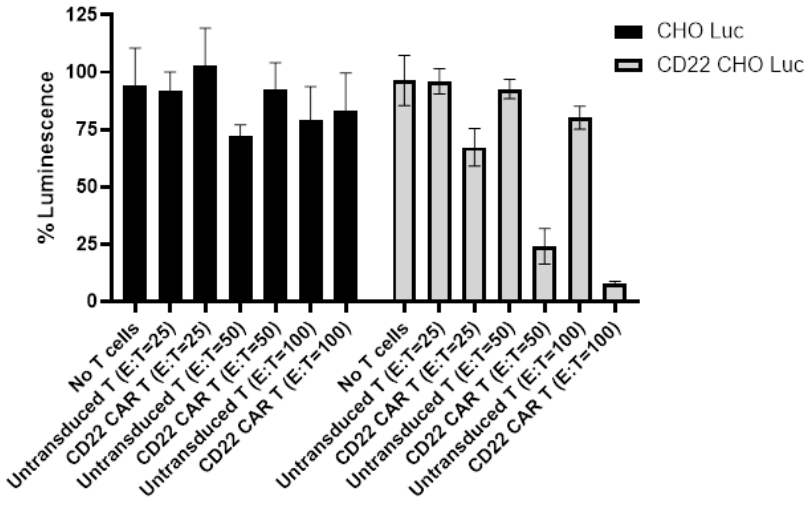

Figure 4. Luciferase-based cytotoxicity assay using CD22 / Luciferase - CHO Recombinant Cell Line as the target cells.
Anti-CD22 CAR-T cells and control untransduced T cells (#78170) were thawed for 24 hours. Anti-CD22 CAR-T cells (effector) were then co-cultured with CD22 / Luciferase - CHO cells (target) for 24 hours at the indicated effector:target (E:T) ratio. The lysis of target cells was determined by measuring luciferase activity. Untransduced T cells (#78170) and Firefly Luciferase CHO Cell Line (#79725) were used as negative controls.

Data shown is representative. For lot-specific information, contact BPS Bioscience, Inc. at support@bpsbioscience.com

References
Schultz L.M., *et al.*, 2024 *Leukemia* 38(5):963-968.
Matthew J.F., *et al.,* 2024 *The Lancet* 404 (10450): 353-363.

Warnings
Donors have been screened and determined negative for:
- Hepatitis B (anti-HBc EIA, HBsAg EIA)
- Hepatitis C (anti-HCV EIA)
- Human Immunodeficiency Virus (HIV-1/HIV-2 plus O)
- Human T-Lymphotropic Virus (HTLV-I/II)
- HIV-1/HCV/HBV
- West Nile Virus
- Trypanasoma cruzi

Note: *Testing cannot guarantee that any sample is completely virus-free. These cells should be treated as potentially infectious and appropriate Biological Safety Level 2 (BSL-2) precautions should be used.*

Troubleshooting Guide
Visit Cell Line FAQs for more information.
For further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Anti-CD19 CAR-T cells	78171	1 vial
CD19 / Firefly Luciferase - CHO Recombinant Cell Line	79714	2 vials
Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ)	78600	50 µl
Anti-BCMA CAR Lentivirus (Clone C11D5.3 ScFv-CD8-CD28-CD3ζ)	78655	50 µl
Anti-CD20 CAR Lentivirus (Clone Leu-16 ScFv-CD8-4-1BB-CD3ζ)	78606	50 µl
Anti-CD22 CAR Lentivirus (Clone m971 ScFv-CD8-4-1BB-CD3ζ)	78608	50 µl

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