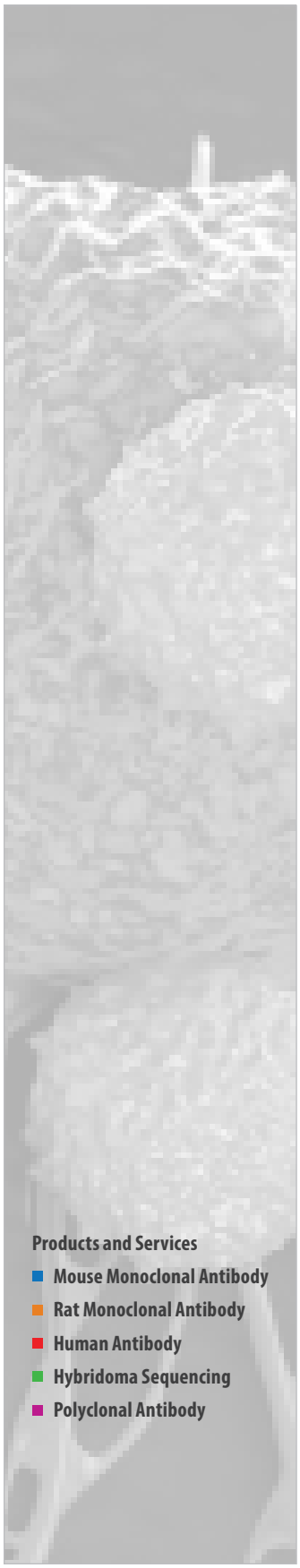
ProMab Biotechnologies, Inc.

CD19-NK CELLS

PM-NK-1001



Products and Services

- Mouse Monoclonal Antibody
- Rat Monoclonal Antibody
- Human Antibody
- Hybridoma Sequencing
- Polyclonal Antibody

DESCRIPTION

The CD19-NK cells are available at $1x10^6$-$1x10^9$ cells in frozen vials, and shipped on dry-ice. The CD19-NK cells were cryopreserved in the next-generation cryopreservation CS10 CryoStor medium (*Sigma Aldrich*, Catalog Number: C2874. CryoStor is a registered trademark of BioLife Solutions, Inc.).

STORAGE TEMPERATURE

Liquid nitrogen vapor phase for frozen vial (-130°C).

CD19-CAR-T CELLS

CD19 is a cell surface marker for lymphocytes that is present on most B cell malignancies, including acute lymphoblastic leukemia (ALL) and various subtypes of non-Hodgkin lymphoma (NHL), including diffuse large B-cell lymphoma (BCL).

CD19-NK cells contain CAR construct with CD19 ScFv (Kochenderfer et al, *J Immunother*, 2009), linked to CD28 costimulatory endodomain in addition to the T-cell receptor derived CD3-zeta intracellular signaling domain. The published 2nd generation antiCD19 single chain antibody (ScFv)-CD28-CD3-zeta CAR construct was used for generation of CD19-CAR-T cells (Kowolik *et al, Can Res., 66*, 2006). The fusion protein was cloned into a lentiviral expression construct (due to a increased safety profile), transduced into NK-92 cells (ATCC, CRL-2407) and then examined for expression of the CD19-CAR fusion protein by Flow cytometry and for cytotoxicity assay with CD19-Hela cells, IFN-gamma and IL-2 assays.

The CAR-NK cells are provided frozen in vials (sterile), shipped on dry ice, and can be used for different assays or activated expanded for different applications. The non transduced and mock control no ScFv-CAR NK cells, lentivirus vector-transduced NK cells are available for ordering as controls for experiments.

APPLICATIONS

To test cytotoxic activity with different cancer cells lines expressing CD19 antigen. To test immune modulators: activators or inhibitors using CD19-CAR NK (effector) and hematological cancer (target) cells. To test different agents, checkpoint inhibitor modulators (Ab, siRNA or small molecules) to activate/expand/freeze and regulate CAR-T cell activity. Different subsets of CD19-CAR-NK cells can be isolated and studied in multiple functional assays.

PROTOCOL

1. Thaw CD19-CAR-NK cell samples quickly in a 37°C water bath until all visible ice has melted. Thaw time for a 1 ml sample in a cryovial is 2-3 minutes. Cryovials should be cool to the touch when removed from the water bath.

2. Dilute cell/CryoStor mixture immediately with CD19-CAR-NK cell culture medium. This can be performed in a single step. The dilution medium should be between 20–37°C. A dilution ratio of 1:10 (sample:medium) or greater is recommended.

3. Plate cells appropriately according to the experimental conditions of assays.

4. Culture the CD19-CAR NK cells or use immediately

Note: T cells that are used immediately after thawing have the highest level of viability of any thawed cells.

CD19 NK cell (CD19-28-ζ) NK cell Prices

Available Cell Numbers	Price	Catalog Number
1x10^6 cells	$1,000	PM-NK 1001-1M
2x10^6 cells	$1,500	PM-NK 1001-2M
1x10^7 cells	$4,000	PM-NK 1001-10M
1x10^8 cells	$8,000	PM-NK 1001-100M
1x10^9 cells	(please request)	PM-NK 1001-1000M

DATA

Real-time cytotoxicity assay with CD19-NK and Hela-CD19 cells

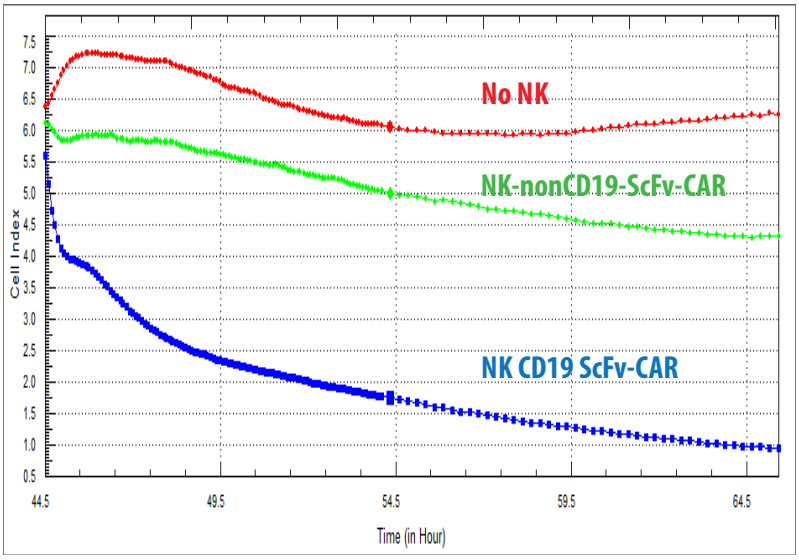


Figure 1 shows high cytotoxic activity of CD19-NK cells with Hela-CD19 cells as a target by Real-time cytotoxicity assay.

REFERENCES

Kochenderfer,J.N., Feldman,S.A., Zhao,Y., Xu,H., Black,M.A., Morgan,R.A., Wilson,W.H. and Rosenberg,S.A. Construction and preclinical evaluation of an anti-CD19 chimeric antigen receptor. *J. Immunother*. 32 (7), 689-702 (2009).

Kowolik CM, Topp MS, Gonzalez S, Pfeiffer T, Olivares S, Gonzalez N, Smith DD, Forman SJ, Jensen MC, Cooper LJ. CD28 costimulation provided through a CD19-specific chimeric antigen receptor enhances in vivo persistence and antitumor efficacy of adoptively transferred T cells. *Cancer Res, 66,* 10995-10004, (2006)

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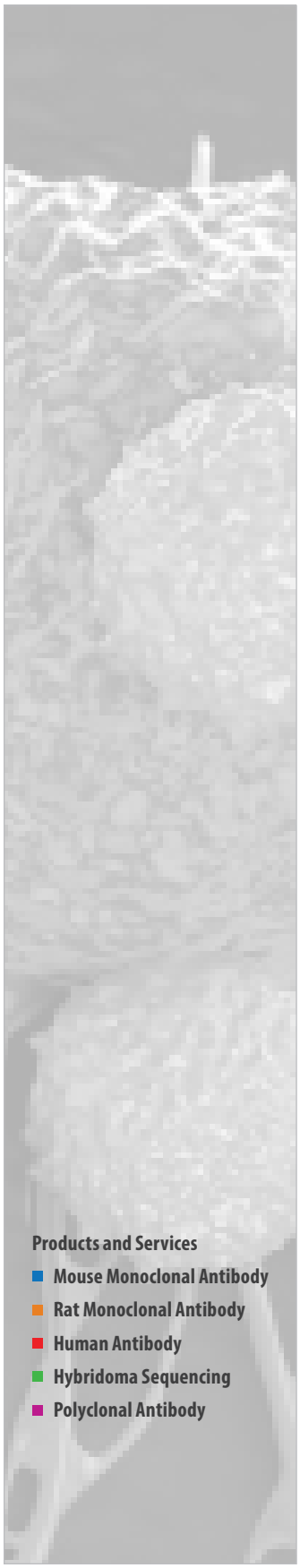

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