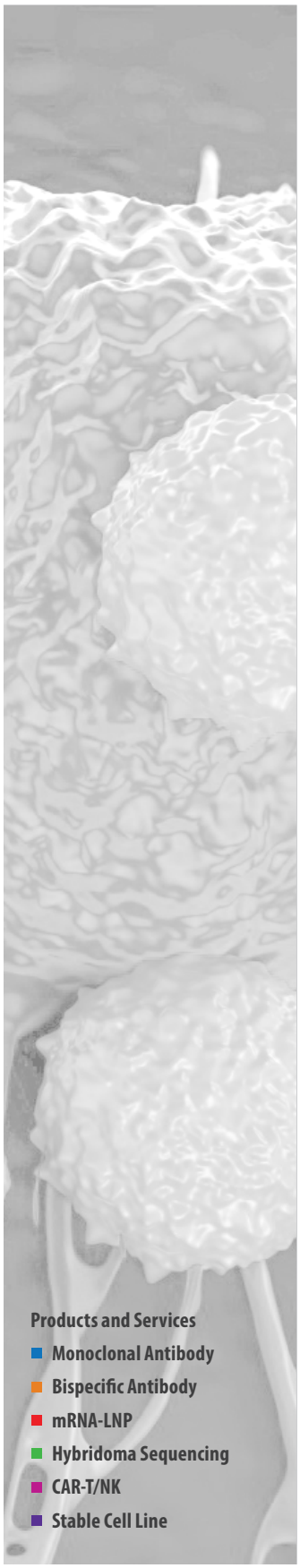
ProMab Biotechnologies, Inc.

Cas9-HA mRNA-LNP

Ready-to-use lipid nanoparticles



Order Information

Catalog#	Size	GenPept No.
PM-LNP-0025	200uL	

Description

Cas9 (CRISPR-associated protein 9) is a 160 kD protein that plays an important role in some bacteria to defend against DNA viruses and plasmids, and is heavily used in genetic engineering applications as known as “CRISPR-Cas9 genome editing”. Cas9 has a two-lobed structure, with the guide RNA positioned between the alpha-helical lobe and the nuclease lobe. The two lobes are helically connected by a single bridge. There are two nuclease domains in the multidomain nuclease lobe, the RuvC that cleaves the non-target DNA strand and the HNH nuclease domain that cleaves the target strand of DNA. CRISPR/Cas9 gene editing enables scientists to edit genomic DNA sequences, adding, deleting or altering them. This product is designed as a tool for the delivery and expression

Composition

mRNA-LNPs suspended in PBS (-Ca, -Mg) (pH: 7.0-7.4).

Translated Protein sequence

Available up request

ProMab Biotechnologies, Inc. | 2600 Hilltop Drive, Building B, Suite C320, Richmond, CA 94806 USA
Toll free 1-866-339-0871 | 1-510-860-4615 (Sales) | 1-510-860-4614 (Technical Support) | Fax 1-510-740-3625 | info@ProMab.com | www.ProMab.com

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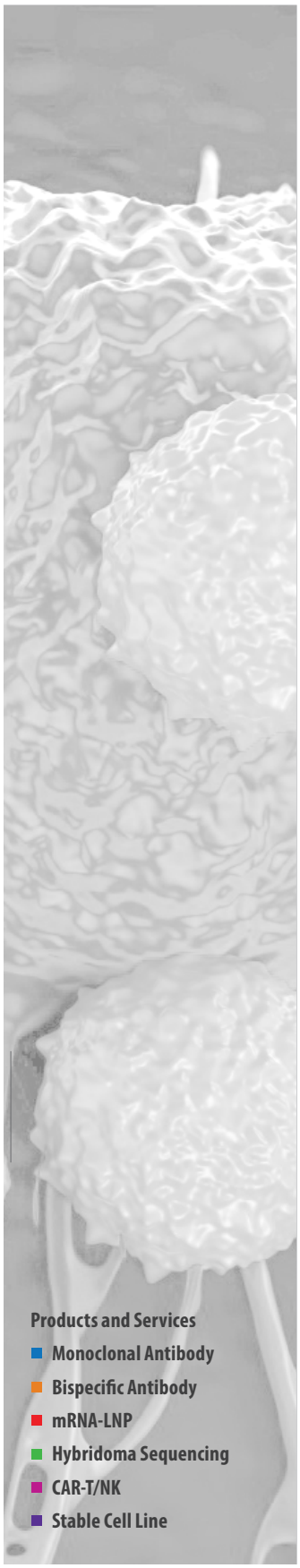

Storage

Product is delivered on blue ice. Store at 4°C for up to 3 months.

Application & Handling

Upon receiving product, briefly pulse spin before opening to ensure product is at bottom of container. It is important not to spin for too long as this may rupture mRNA-LNPs. Do not vortex. Work with mRNA-LNPs on ice and minimize the time that the product spends at room temperature. After handling the product during experiments, return immediately to ice. mRNA-LNP products should only be handled with certified RNase-free reagents and consumables. Use of filtered pipette tips is highly recommended.

Safety & Research Disclosure

All ProMab mRNA lipid nanoparticle products are for in vitro research use only. Products are not FDA approved for human use.

General Protocol

1. Prior to transfection: Plate 1ml of cells at a density of [1.0E6 cells/ml] in a single well of a 12-well culture plate. Ensure the cells you are using are viable and healthy. Try not to let your cells sit for longer than 5 minutes prior to transfection. Cell clumping at the time of transfection may reduce transfection efficiency. *Note: If cell clumping occurs, gently pipette your culture up & down to ensure you have a single cell suspension before transfecting.

2. Briefly pipette mRNA-LNP mix up & down to resuspend. Add 20-40ul of the mRNA-LNP product dropwise directly to your 1ml culture. Gently tilt plate back and forth to mix (not necessary if you are using cells which will be immediately placed back into a shaker). Place your transfected cells back into their original culture conditions.

3. Check cell expression by FACS or by using other detection methods at 24hr intervals after transfection. *Note: This is a generalized protocol for transfection using mammalian suspension culture cells. Transfection volume may be scaled up or down proportionately using the volumes given. HEK-293s cells were grown and transfected in FreeStyle™ F17 Expression Medium (Gibco, Cat#: A1383501), supplemented with GlutaMAX™ (Gibco, Cat#: 35050061), and Poloxamer 188 Non-ionic Surfactant (Gibco, Cat#: 24040032). T-cells were grown and transfected in a culture medium supplemented with 10% FBS (Omega Scientific, Cat#: FB-02). When transfecting cells using mRNA-LNPs, it is typically necessary for the cell culture medium to be supplemented with 10% FBS at the time of transfection. Without this supplement, transfection efficiency will drop significantly. For mRNA-LNP transfection of cells which cannot use FBS in the culture medium, please contact us at (510) 860-4615. Alternative transfection methods are available.

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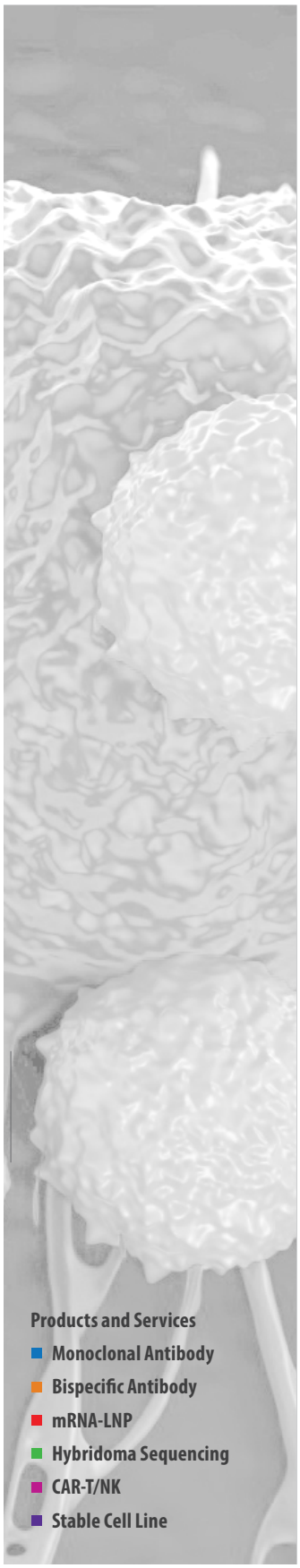

Products and Services

- Monoclonal Antibody
- Bispecific Antibody
- mRNA-LNP
- Hybridoma Sequencing
- CAR-T/NK
- Stable Cell Line

CAR9 Functional Knockout in Human T Cells Transfected with PM-LNP-0025

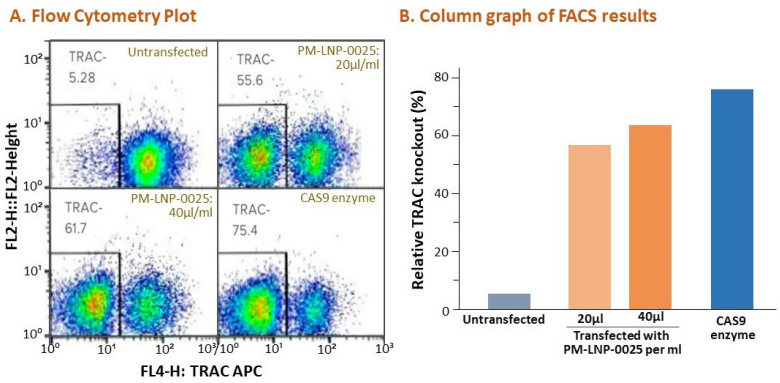

Figure 1. PM-LNP-0025 nanoparticle-treated HEK293S cells express functional Cas9 protein, indicated by the disappearance of the TCRab complex from human T cells electroporated with TRAC sgRNA. Cas9-mediated knockout of the TRAC locus prevents the cells from synthesizing new TCRab complexes to replace those that are removed by protein turnover. The graph on the right shows that the extent of TRAC locus knockout was almost as high as that achieved by

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