



CAR-T Cell Thawing Protocol

For Catalog #: PM-CAR series.

Revised March 2022

These cells have been frozen in Cryo-SFM Medium (Sigma, Catalog Number: C-29912). Please transfer the vial of frozen cells into liquid nitrogen upon receipt without allowing the cells to thaw prematurely.

Please note: These CAR-T cells were generated by transduction of activated T cells with a CAR-encoding lentivirus. Once thawed, the cells are ready to be used in functional assays. If necessary, the cells may be cultured for several days in CAR-T medium* containing human IL-2 before functional analysis.

Thawing Procedure

For each vial of frozen cells:

1. Pipet 5 ml of CAR-T medium* (or any culture medium containing 5-10% serum) into a 50-ml centrifuge tube and partially immerse the tube in a 37°C water bath.
2. Partially immerse the vial of frozen CAR-T cells in the 37°C water bath and gently agitate the vial until the residual frozen sphere is ~3 mm in diameter. The label on the vial can be removed to help you visualize the size of the frozen sphere.
3. Using a manual pipettor, transfer 0.5 - 1 ml of warm medium from the 50-ml tube into the vial of cells, then immediately pipet the contents of the vial back into the 50-ml tube containing the warm medium.
4. Centrifuge the 50-ml tube at 300g for 5 minutes at room temperature.
5. Discard supernatant and re-suspend cell pellet with pre-warmed medium.
 - a. If performing a functional assay, proceed to step 8.
 - b. If culturing the cells, proceed to step 6.
6. Suspend the cells in warm CAR-T medium* containing IL-2 at a density of $0.5-1 \times 10^6$ cells/ml. The cell density can be measured using a hemacytometer with trypan blue.
7. Transfer the cells to an appropriately sized tissue culture vessel and store the vessel in a humidified 37°C incubator with 5% CO₂ for several days. The cells should start to recover during this time.
8. Suspend the cells in assay medium to the desired density. The cell density can be measured using a hemacytometer with trypan blue. Perform the assay**.

* ProMab has CAR-T medium available for purchase: # PM-CAR2000 (with human serum) or PM-CAR2001 (with FBS). Human IL-2 must be added to the medium before use.

** For cytotoxicity assays, ProMab recommends suspending the CAR-T cells at 1×10^6 cells per ml in RPMI-1640 medium containing 10% FBS. The cells can be cultured with target cells (in the same medium) at effector:target ratios of 1:1 to 10:1 for 4-24 hours, after which target cell killing is determined.