



# CAR-T Cell Thawing Protocol

For Catalog #: PM-CAR series.

Revised June 2019

These cells have been frozen in CryoStor 10 Medium (Sigma, Catalog Number: C2874). Please transfer frozen cells to liquid nitrogen upon receipt without allowing contents to thaw prematurely.

Please note: These CAR-T cells have been transduced with a lentivirus containing the chimeric antigen receptor cassette, and are fully activated and amplified. Once thawed, they are ready to be used in an assay. Therefore, it is suggested that you have the experiment set up prior to thawing the cells. The cells may be maintained for up to 4 weeks in CAR-T medium\* containing 300IU/mL of human IL2 (Invitrogen). However, this is not recommended.

## **Thawing Procedure**

For each vial of frozen cells:

1. Thaw cells quickly (with gentle agitation) in a 37°C water bath until all contents are completely thawed.
2. Wash cells immediately with 10 ml of pre-warmed tissue culture medium\*.
3. Mix well and centrifuge at 300g for 5 minutes at room temperature.
4. Discard supernatant and re-suspend cell pellet with pre-warmed medium.
5. Culture the cells in culture medium for a minimum 6-8 hours or overnight before use in your assay.
6. Determine cell concentration. Depending on the application, cells are diluted to  $1 \times 10^4$  or  $1 \times 10^5$  per ml.

The cells are now ready to be used in your assay.

\* ProMab has CAR-T medium available for purchase, Cat. # PM-CAR2000.