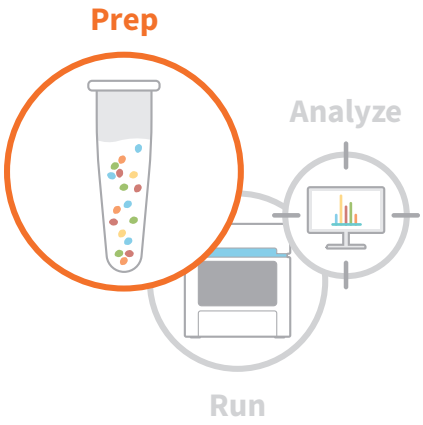


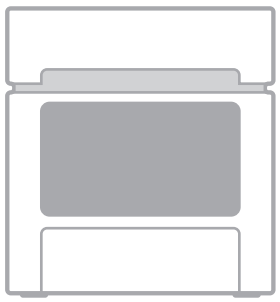

# CAR-T Protocol: antigen specific stimulation with transduced APCs

Ensure you achieve the maximum benefit from our systems and generate useful data ASAP that relates to in vivo immune activity

## Sample Prep

Sample Prep for IsoCode

## IsoLight Automation



IsoLight Automated Run

## Data Analysis

IsoSpeak Software Analysis

isoplexis

www.isoplexis.com

## Prep, Run, Analyze

### Day 1 : Recovery and Preparation

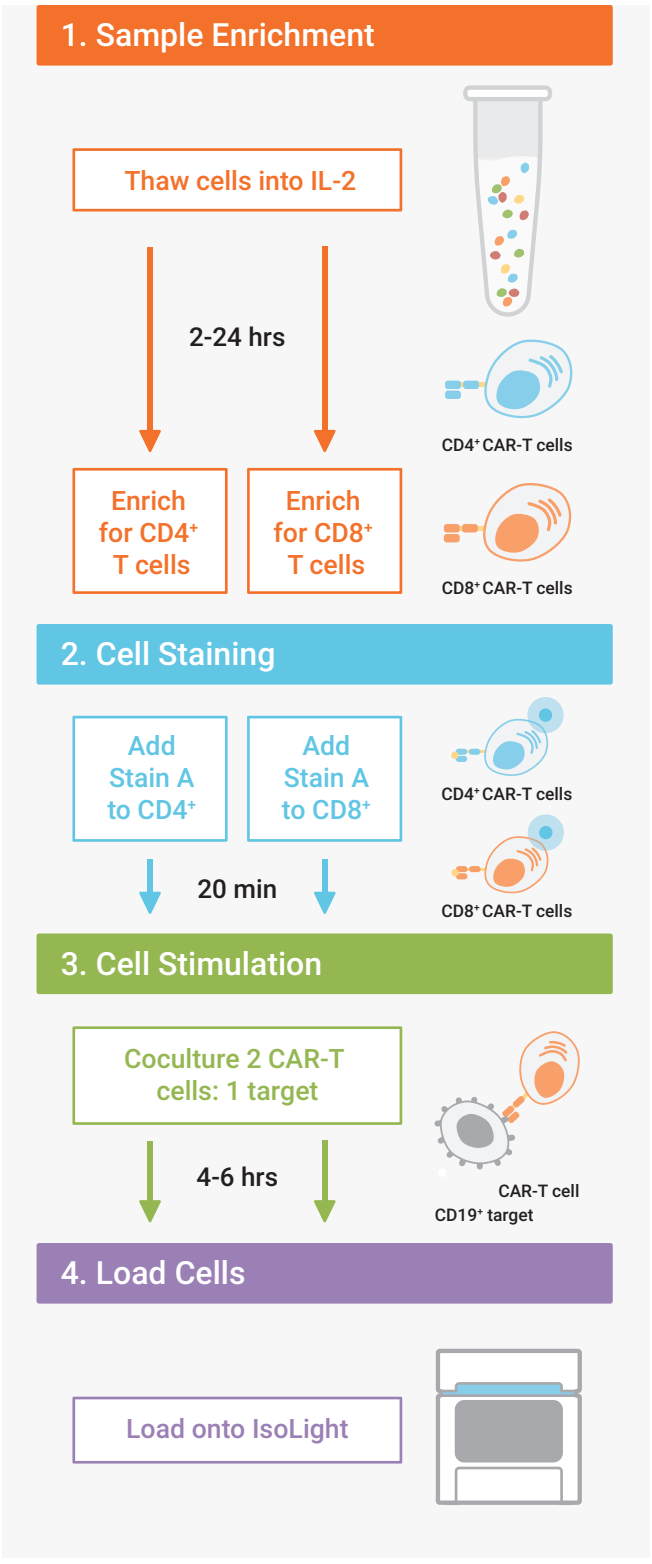
- ☐ Thaw CAR-T cells and APC targets
- ☐ Suspend cells in complete RPMI media with IL-2 (10 ng/ml) at a density of 1-5 x $10^6$ cells/ml
- ☐ Recover cells at 37°C, 5% $CO_2$, O/N

### Day 2 : Sample Enrichment

- ☐ Check cell viability and deplete dead cells by Ficoll or low speed spin if needed (viability<80%)
- ☐ Enrich $CD4^+$ and $CD8^+$ CAR-T cells with anti-CD4 and anti-CD8 beads respectively (Miltenyi kit)

### Day 2 : Cell Staining

- ☐ Stain $CD4^+$ CAR-T cells with **Stain A** and $CD8^+$ CAR-T cells with **Stain A** (if using general stain)
- ☐ Centrifuge enriched cell fractions at 300xg (RCF) for 10 min and resuspend in 1000 uL of PBS.
- ☐ Add 1µL (1:1000 final dilution) of Stain 'A' to cells and incubate for 10 minutes at 37°C in the dark.
- ☐ After 10 minutes, add 5mL of complete RPMI and incubate for 10 minutes at 37°C in the dark.
- ☐ Centrifuge stained cells at 300xg for 10 minutes and resuspend in 500 uL of complete RPMI.
- ☐ Take an aliquot of cells to count (10µL). Spin the enriched cell fractions at 300xg (RCF) for 10 minutes.
- ☐ Aspirate the supernatant and resuspend the T cells in complete RPMI. Keep cells in the incubator at 37°C until use.
- ☐ Resuspend $CD4^+$ and $CD8^+$ CAR-T cells in complete RPMI media at a cell density of 2 x $10^6$ cells /ml



# Prep, Run, Analyze

## Day 2 : Cell Stimulation

- ☐ Prepare positive and negative APC targets (e.g., transduced K562) at a cell density of 1 x $10^6$ cells/ml
- ☐ **Coculture 2 CAR-T cells :1 APC target** in a well of a U-bottom 96-well plate (50uL of target suspension and 50uL of CAR-T cell suspension) as follows:
- ☐ $CD4^+$ CAR-T : positive targets ( e.g., CD19 transduced K562)
- ☐ $CD4^+$ CAR-T : negative targets ( e.g., NGFR transduced K562)
- ☐ $CD8^+$ CAR-T : positive targets ( e.g., CD19 transduced K562)
- ☐ $CD8^+$ CAR-T : negative targets ( e.g., NGFR transduced K562)
- ☐ Incubated for **4-6 hours** at 37 C, 5% $CO_2$

## Day 2 : Load Cells

- ☐ Optional: Collect supernatants (100 ul per well) from all groups and store at -80°C if customer wishes to store or use for population control.
- ☐ Resuspend $CD4^+$ and $CD8^+$ CAR-T cells in complete RPMI media at 1.0 x$10^6$ cells/ml.
- ☐ Load 30uL of $CD4^+$ CAR-T cell suspension into an IsoCode chip at a density of 1.0 x $10^6$ cells/ml Load IsoCode chips onto IsoLight System.
- ☐ Load 30uL of $CD8^+$ CAR-T cell suspension into an IsoCode chip at a density of 1.0 x $10^6$ cells/ml. Load IsoCode chips onto IsoLight System.