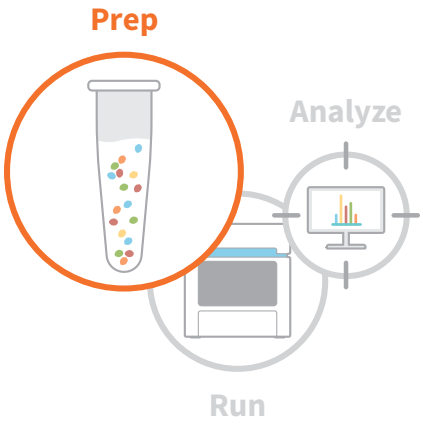


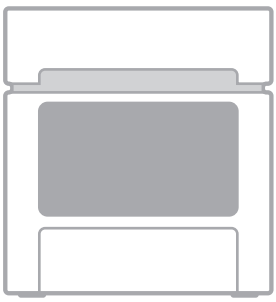
PBMC Protocol: T cell stimulation with CD3/CD28

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

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Prep, Run, Analyze

Day 1 : Recovery and Preparation

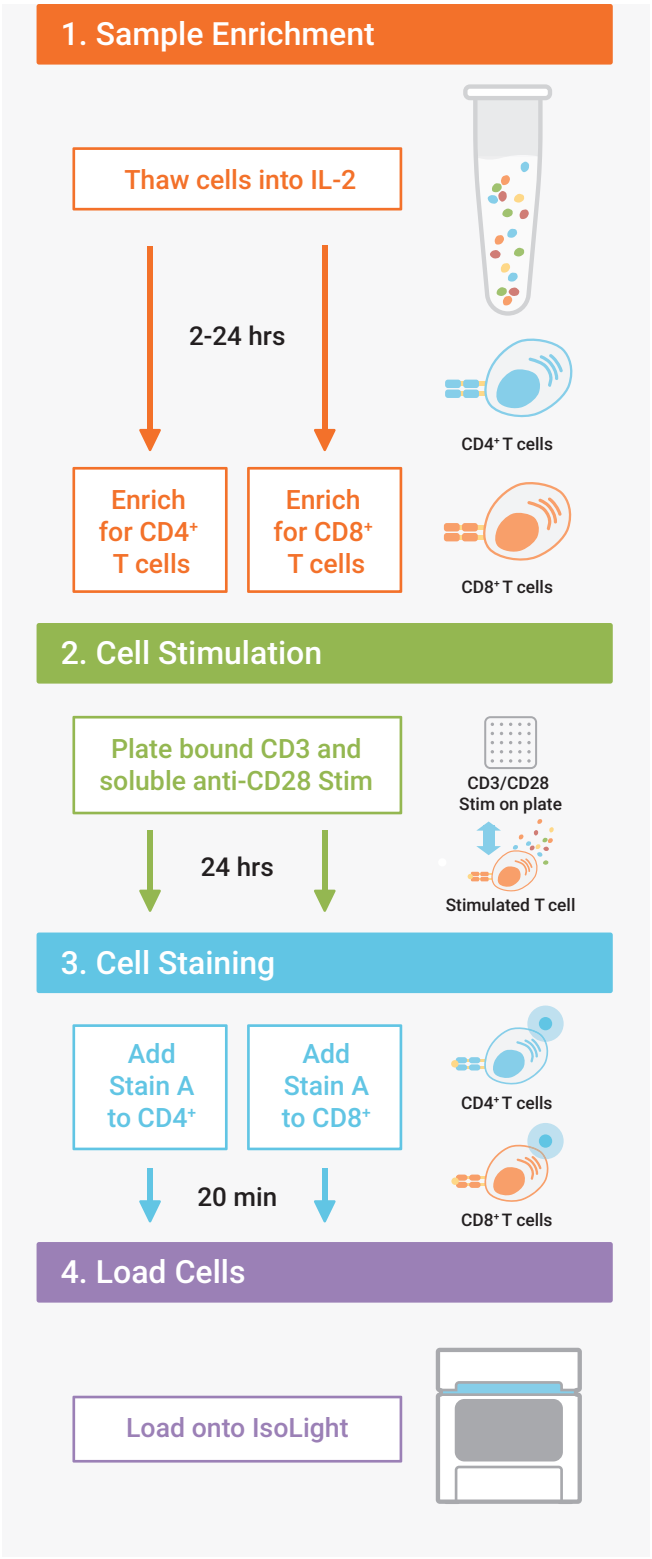
- ☐ Thaw PBMCs or purified T cells
  Suspend cells in complete RPMI media with IL-2 (10 ng/ml) at a density of 1-5 x 10^6 cells /ml
- ☐ Recover cells at 37°C, 5% CO_2, O/N
- ☐ Coat wells with **anti-human CD3 (10 ug/ml in PBS, 100 ul/well)** in a 96-well flat-bottom plate at 4°C, O/N

Day 2 : Sample Enrichment

- ☐ Check cell viability and deplete dead cells by Ficoll or low speed spin if needed (viability<80%)
- ☐ Enrich $CD4^+$ and $CD8^+$ T cells with anti-CD4 and anti-CD8 beads respectively (Miltenyi kit)
- ☐ Resuspend $CD4^+$ and $CD8^+$ T cells in complete RPMI media at a cell density of 1 x 10^6 cells/ml

Day 2: Cell Stimulation

- ☐ Seed 30- 200 ul of $CD4^+$ and CD8+ T cell suspension into a well in the 96-well flat-bottom plate with **soluble anti-human CD28 (5 ug/ml)**
- ☐ Incubate the plate at 37°C, 5% CO_2 for **24 hours**



Prep, Run, Analyze

Day 3: Cell Staining

- ☐ 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.
- ☐ Collect $CD4^+$ and $CD8^+$ T cells by up and down pipetting
- ☐ Stain $CD4^+$ T-cells with **Stain A** and $CD8^+$ T cells with **Stain A**
- ☐ 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 $CD8^+$ T cells in complete RPMI media at 1.0 $x10^6$ cells/ml.

Day 3: Load Cells

- ☐ Load 30uL of $CD4^+$ T cell suspension into an IsoCode chip at a density of 1.0 x 10^6 cells/ml.
- ☐ Load 30uL of $CD8^+$ T cell suspension into an IsoCode chip at a density of 1.0 x 10^6 cells/ml.
- ☐ Load IsoCode chips onto IsoLight System.