

Assay Name: CIK-mediated ADCC using CFSE and PI

Assay ID: Celigo_01_0008

Description: Measure CIK-mediated ADCC by counting total CFSE positive/PI negative live tumor cells

Stains: CFSE (green total cells); Propidium Iodide (red dead cells)

Imaging channels: Bright field, Green, Red

Image analysis algorithm: Celigo software Target 1 + 2 + 3

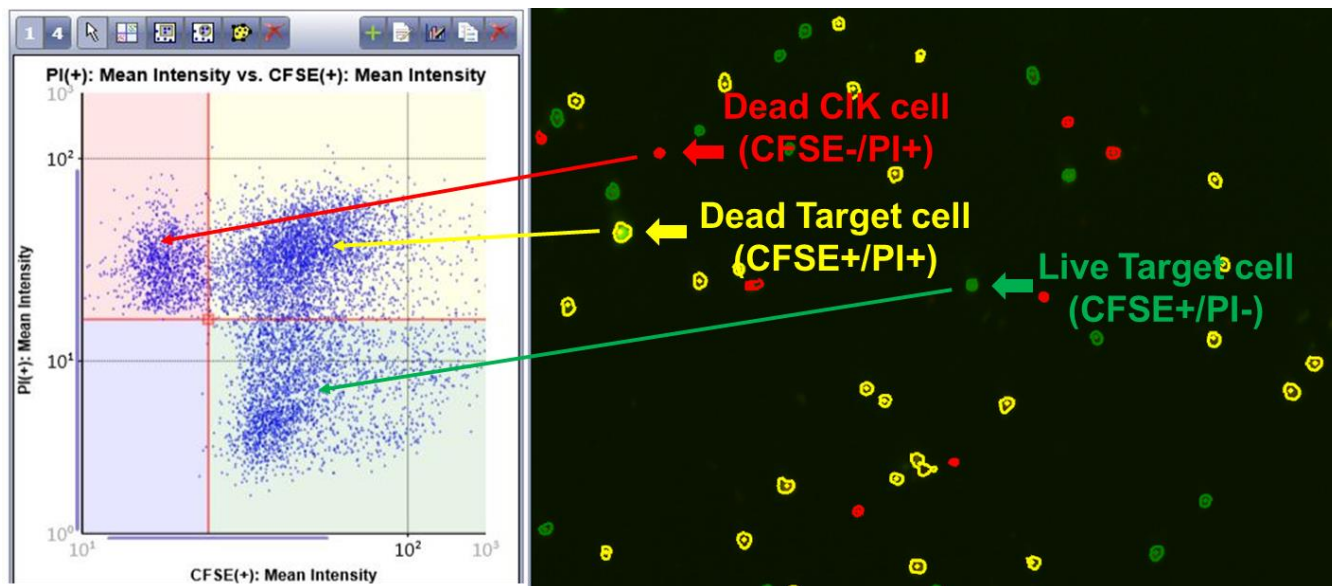
Methods:

1. Culture and collect Target cells and stain with CFSE
2. Seed the Target cells in the wells of 96-well microplate
3. Add the CIK Effector cells and titrations of antibodies
4. Co-culture the Target cells with cultured CIK Effector cells with antibodies for 24 hours and observe the CIK ADCC
5. Stain the cells in the well with PI to identify the dead cells (Nexcelom, Cat# CS1-0109)
6. Use Celigo and capture images at Target cells over time
7. Use the equation to calculate cytotoxicity

$$a. \text{ Cytotoxicity \%} = \frac{\text{Dead Target Count}}{\text{Dead+Live Target Count}}$$

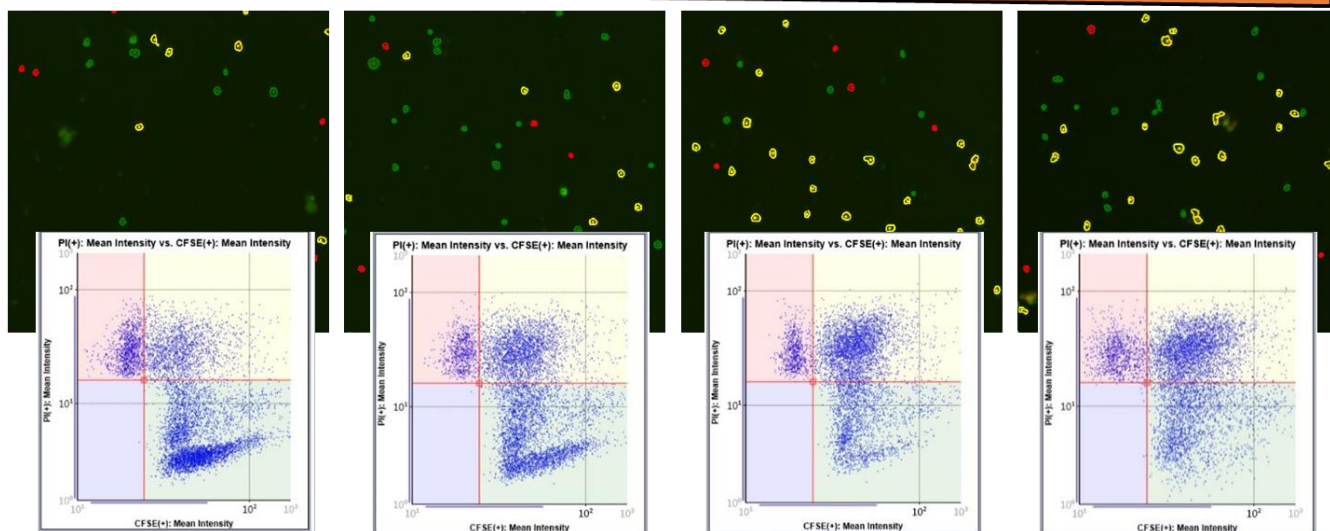
Results:

CIK-mediated ADCC cytotoxicity detection method



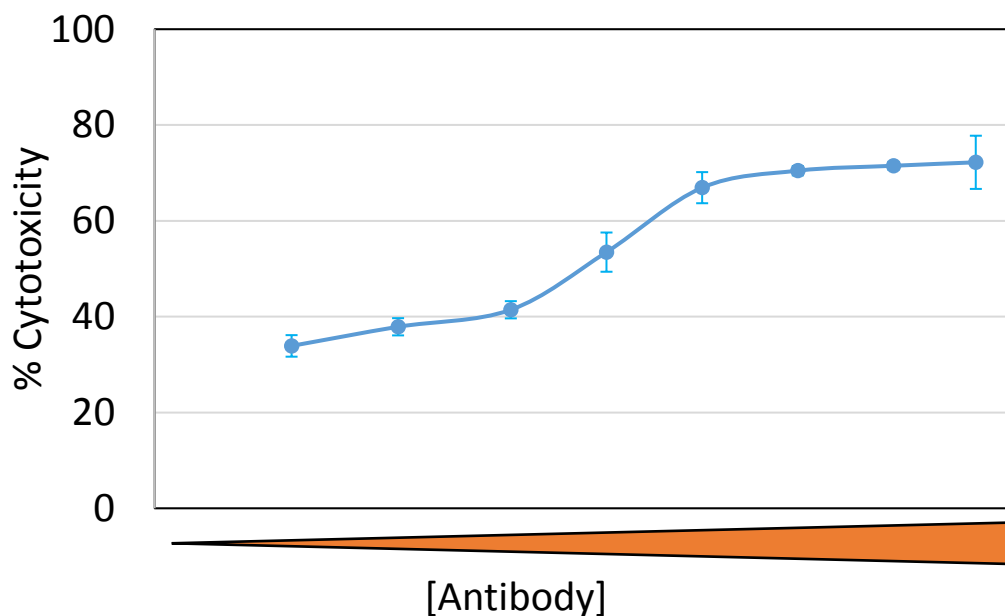
- Direct visualization of cell classification based on flow-like gating

[Antibody]



- Visually, number of dead Target cells (Yellow), increased as the [antibody] increased

CIK-mediated ADCC dose-dependent results



- At the endpoint, a clear cytotoxicity dose response is shown in respect to [antibody]
- The % cytotoxicity is calculated by the equation previously shown
- As the [antibody] increased, the % cytotoxicity also increased