

## Assay Name: Neutrophil-mediated ADCC using calcein AM

**Assay ID:** Celigo\_01\_0003

**Description:** Measure neutrophil ADCC by counting total calcein AM-positive, live tumor cells

**Stains:** calcein AM (green total live cells)

**Imaging channels:** Bright field and green

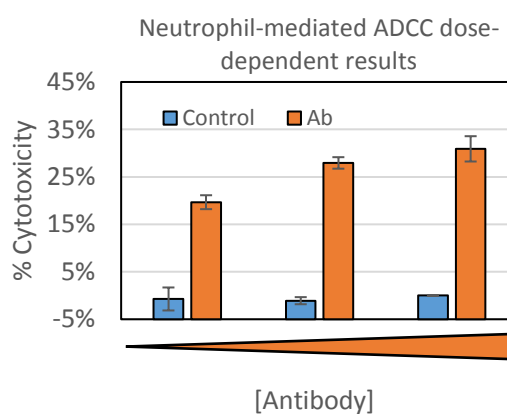
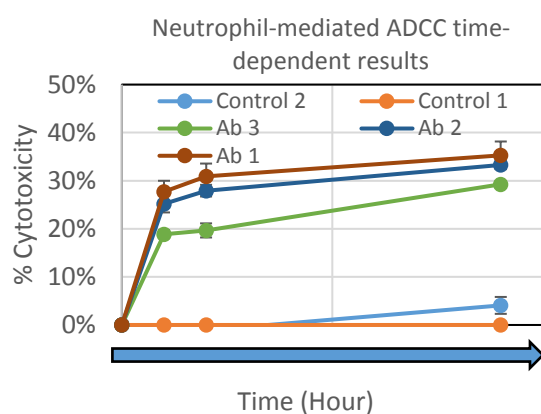
**Image analysis algorithm:** Celigo software Target 1 + 2

### Methods:

1. Culture and collect Target cells and stain with calcein AM (Nexcelom, Cat# CS1-0119)
2. Seed the Target cells in the wells of 96-well microplate
3. Add the neutrophil Effector cells and different concentrations of antibodies
4. Co-culture the Target cells with cultured neutrophil Effector cells with antibodies for 4 hours and observe the neutrophil mediated ADCC
5. Use Celigo to capture images hourly and analyze the total number of calcein AM-positive live Target cells over time
6. Use the 2 equations to calculate cytotoxicity
  - a. Normalized to  $t = 0$ 
    - i.  $\% \text{ Cytotoxicity (normalized to time)} = 1 - \frac{\text{Calcein AM count}_{t=x}}{\text{Calcein AM count}_{t=0}}$
  - b. Normalized to spontaneous release
    - i.  $\% \text{ Cytotoxicity} = \% \text{ cytotoxicity (sample)} - \% \text{ cytotoxicity (spont)}$

### Results:

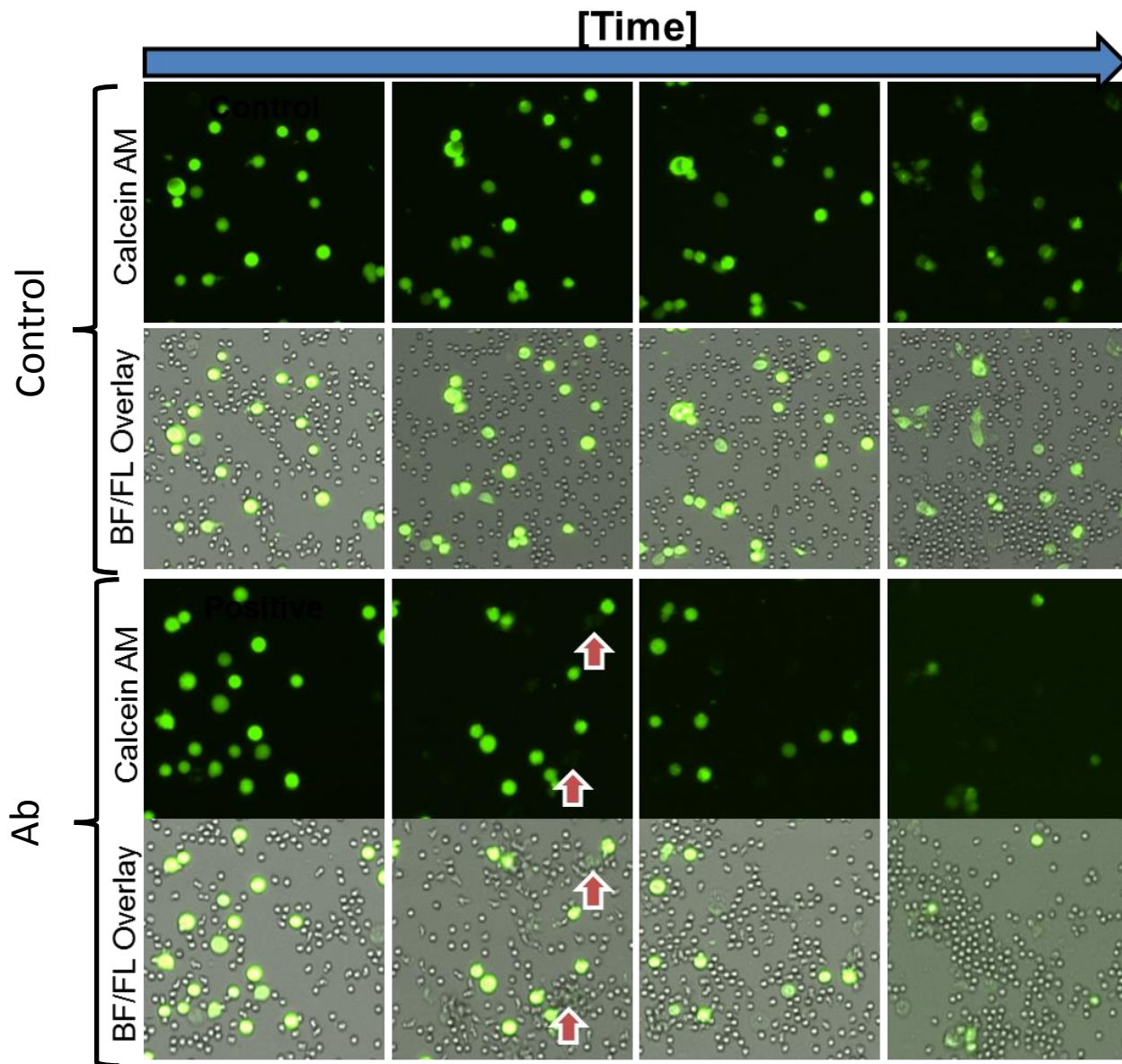
Neutrophil-mediated ADCC time- and dose-dependent results



- As time increased, the Target cell cytotoxicity increased for the positive antibody, while the control did not show significant change

- The dose response showed antibodies induced a high neutrophil-mediated ADCC effect to the Target cells

Neutrophil-mediated ADCC bright field and fluorescent overlay images



- As time increased, the number of calcein AM-stained Target cells decreased
- In the control antibody, the neutrophils did not induce cytotoxicity, where the calcein AM-positive cells remained bright green
- It is clear in the bright field and fluorescent overlay images that the neutrophils elongated and surrounded the Target cells, which induced cytotoxicity (shown with the red arrows)