# **Assay Name:** Complement-dependent cytotoxicity using calcein AM

**Assay ID:** Celigo_01_0004

**Description:** Measure complement-dependent cytotoxicity (CDC) 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

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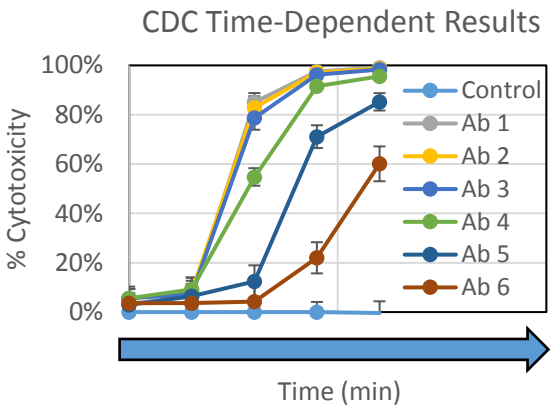
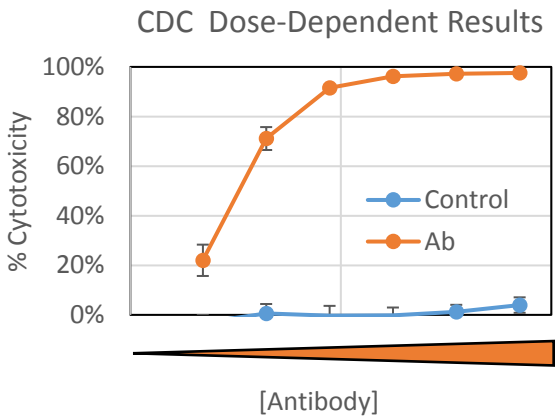
**Methods:**

1. Culture and collect Target cells and stain with calcein AM (Nexcelom, Cat# CS1-01190
2. Seed the Target cells in the wells of 96-well microplate
3. Add different concentrations of antibodies
4. Add serum
5. Use Celigo and capture images hourly and analyze the total number of live Target cells over time
6. Use the 2 equations to calculate cytotoxicity
    a. Normalized to t = 0
        i. $\%\ Cytotoxicity\ (normalized\ to\ time) = 1 - \frac{Calcein\ AM\ count_{t=x}}{Calcein\ AM\ count_{t=0}}$
    b. Normalized to spontaneous release
        i. $\%\ Cytotoxicity = \%\ cytotoxicity\ (sample) - \%\ cytotoxicity\ (spont)$

**Results:**

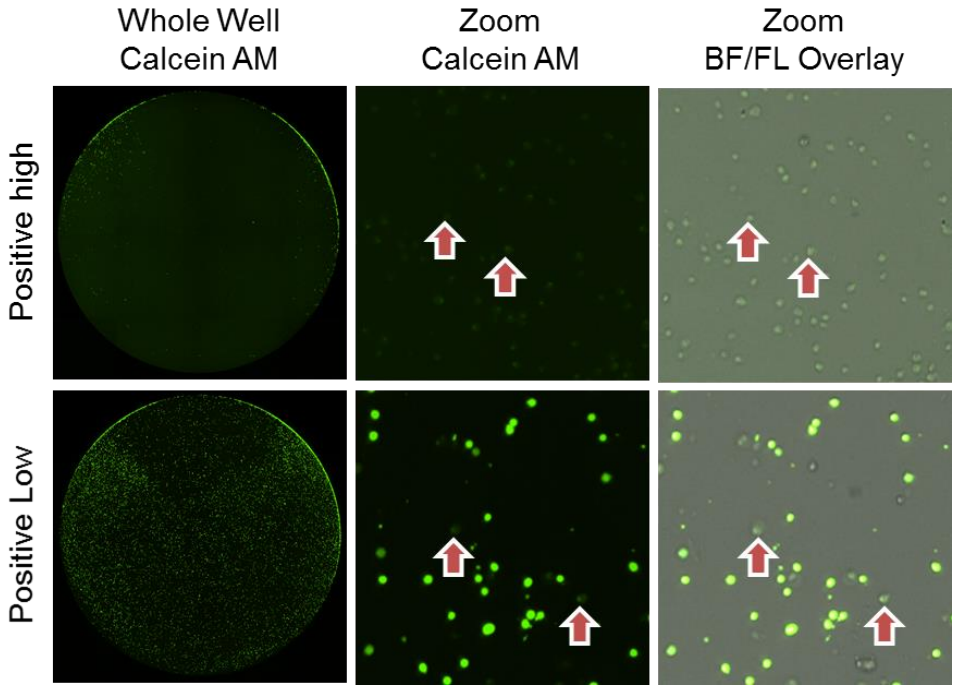
CDC time- and dose-dependent results




- As time increased, the Target cell cytotoxicity increased for the positive antibody, while the control did not show significant change
- The endpoint dose response showed positive antibody-induced high CDC effect to the Target cells

CDC bright field and fluorescent overlay images




- As time and antibody concentration increased, the number of calcein AM-stained Target cells decreased
- In the top three images, where high [antibody], high cytotoxicity is shown, where almost 100% of the cells lost calcein AM fluorescence (indicated with the red arrows)
- In the bottom three images, where low [antibody], lower cytotoxicity is shown, with numerous calcein AM-positive, live tumor cells present (indicated with the red arrows)