

Assay Name: ADC-mediated cytotoxicity using bright field

Assay ID: Celigo_01_0005

Description: Measure antibody drug conjugate (ADC) mediated cytotoxicity by measuring changes in % confluence over time

Stains: Label free

Imaging channels: Bright field

Image analysis algorithm: Celigo software Confluence

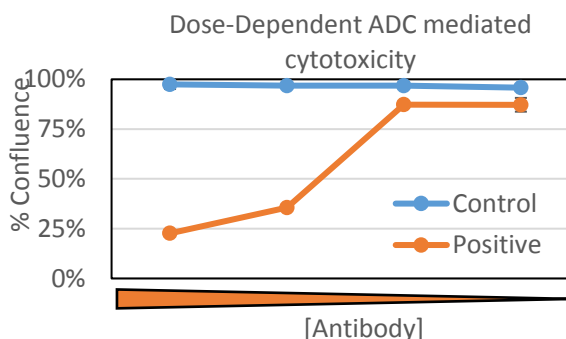
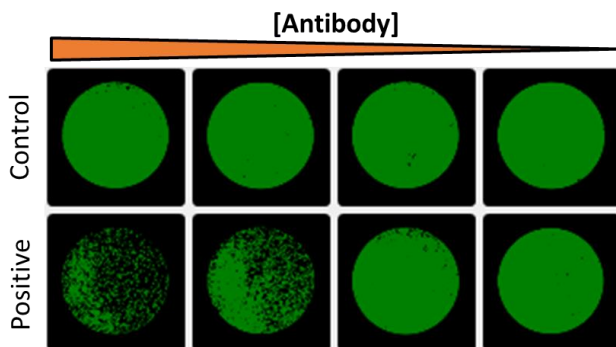
Methods:

1. Culture and collect Target cells
2. Seed the Target cells in the wells of 96-well microplate
3. Add different concentrations of antibody drug conjugates (ADCs)
4. Use Celigo and capture images daily and analyze the % confluence over time
5. Use the equation to calculate cytotoxicity

$$a. \% \text{ Cytotoxicity (control)} = 1 - \frac{\% \text{ Confluence}_{\text{treated}}}{\% \text{ Confluence}_{\text{control}}}$$

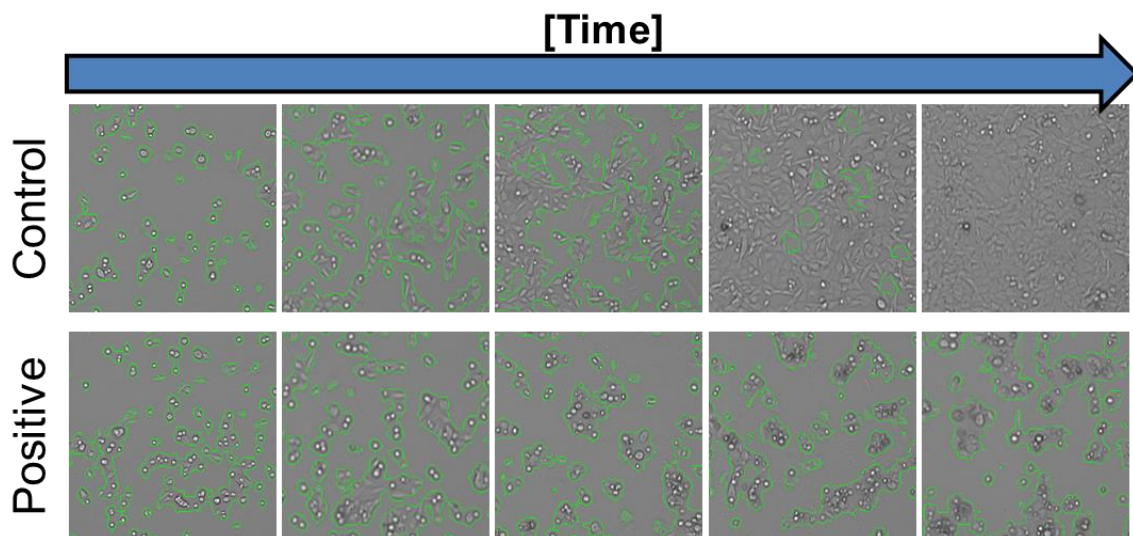
Results:

ADC-mediated cytotoxicity bright field-filled whole well images



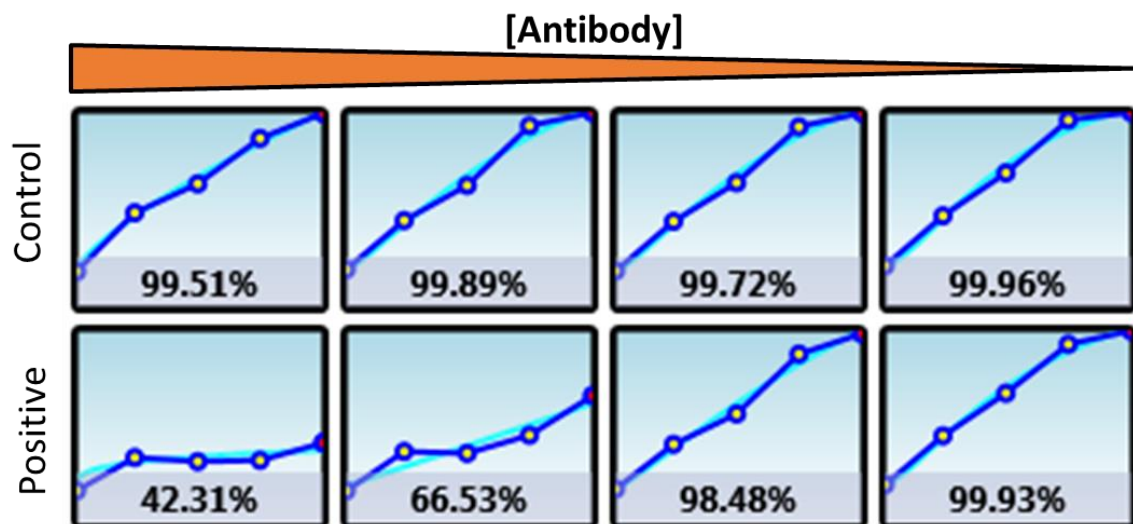
- Cell count and % confluence can be measured using Celigo
- Resulting cell proliferation kinetics based on % confluence can be directly plotted to observe the effect of antibodies on Target cells
- High [antibody], high cytotoxicity and growth inhibition are shown, where the cell area coverage or % confluence are clearly observed in the filled view of the wells

ADC time-dependent bright field images



- The captured images showed increasing in % confluence for the control and positive ADC-mediated cytotoxicity effect on the treated Target cells

ADC time-dependent growth curves generated in Celigo



- The time-course kinetic plots from the Celigo software showed reduction in % confluence over time
- The Control sample showed that at different concentrations of antibody, the growth curves remained consistent
- The positive antibody sample showed slowed growth at the highest antibody dosage