

## Assay Name: NK cell-mediated cytotoxicity using calcein AM

**Assay ID:** Celigo\_01\_0001

**Description:** Measure NK cell direct killing by counting total live tumor cells

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

**Imaging channels:** Bright field and green

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

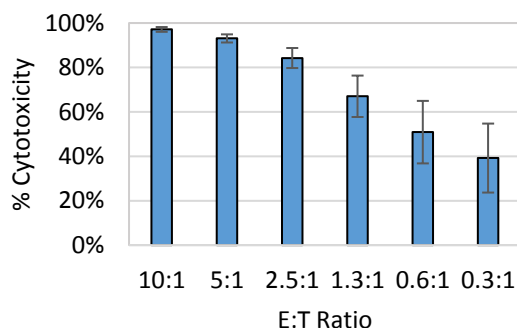
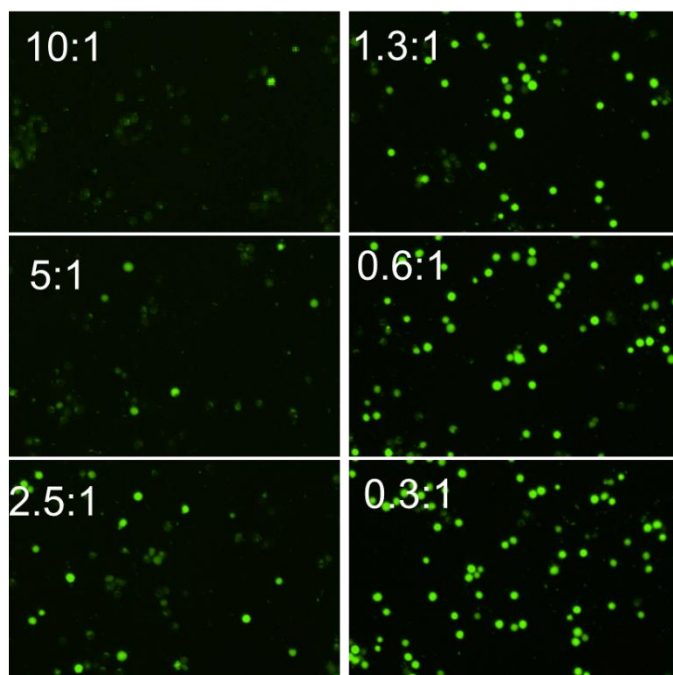
### Methods:

1. Culture and collect K562 and IMR32 Target cells and stain with calcein AM (Nexcelom, Cat# CS1-0119)
2. Seed the Target cells in the wells of a 96-well microplate
3. Add the NK effector cells at E:T ratios of 10:1, 5:1, 2.5:1, 1.3:1, 0.6:1, and 0.3:1
4. Co-culture the K562 or IMR32 cells with cultured NK Effector cells for 4 hours and observe the NK cell-mediated cytotoxicity
5. Use Celigo and capture images hourly and analyze the total number of live Target cells over time
6. Use the equation below to calculate cytotoxicity

$$a. \quad \% \text{ Cytotoxicity} = 1 - \frac{\text{Calcein AM count}_{\text{treated}}}{\text{Calcein AM count}_{\text{control}}}$$

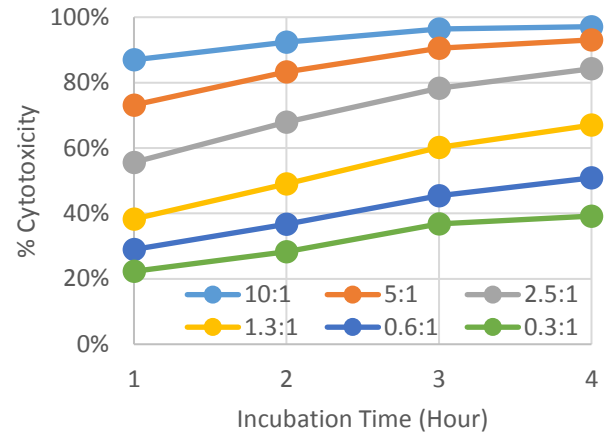
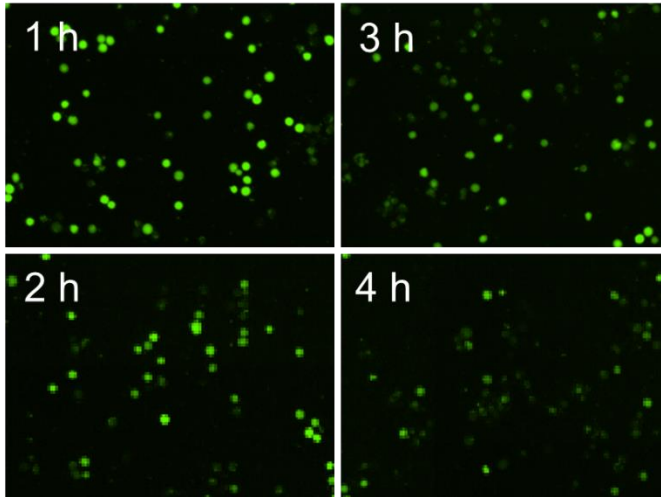
### Results:

E:T ratio-dependent NK cell-mediated cytotoxicity



- The calcein AM fluorescent images showed a reduction in Target cell number as the E:T ratios decreased
- The % cytotoxicity is plotted to show the effect of E:T ratios on Target cell cytotoxicity

## Time-dependent NK cell-mediated cytotoxicity



- As time increased, the number of calcein AM-stained Target cells decreased
- Different E:T ratios showed different NK cell-mediated cytotoxicity effect on the Target cells