

## Assay Name: Endpoint antibody internalization using pHrodo and Hoechst

**Assay ID:** Celigo\_02\_0022

**Description:** Antibody internalisation assays using a pH sensitive dye.

**Stains:** Hoechst and pHrodo (pH sensitive dye)

**Imaging channels:** Blue and Red Fluorescent channels

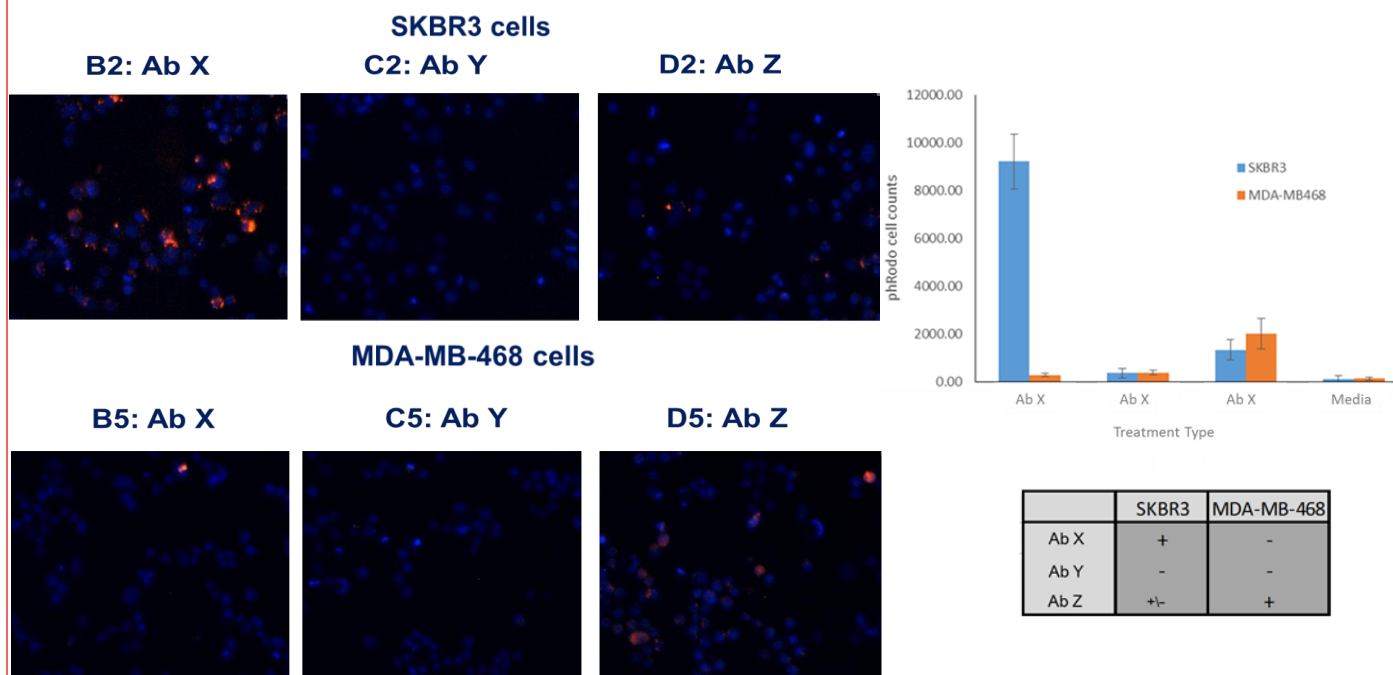
**Image analysis algorithm:** Celigo software Target 1 + Mask

### Methods:

1. SKBR-3 and MDA-MB-468 cells were seeded at 10,000 cells/well and left overnight to adhere
2. pHrodo-labeled antibodies X, Y and Z were added at 300ng/ml and incubated for 24 hours
3. Cells were labeled with 1 µg/ml of Hoechst for 30 minutes and read on the Celigo using Target 1 + Mask

### Results:

Combined fluorescent images and numerical data detecting antibody internalisation



- The qualitative pHrodo red fluorescent images overlays with nuclear Hoechst imaging show cellular localization of antibodies using the pH-sensitive dye.
- The quantitative data in the bar graph confirms the previous known activities of antibodies X, Y and Z.