

Assay Name: Kinetic pHrodo Antibody Internalization using Confluence

Assay ID: Celigo_02_0020

Description: Antibody internalization assays using a pH sensitive dye.

Stains: pHrodo (pH sensitive dye)

Imaging channels: Bright field and Red Fluorescent channel

Image analysis algorithm: Celigo software Confluence ratio

Methods:

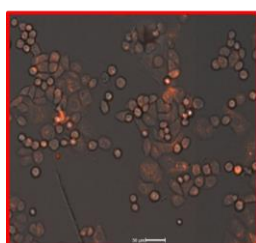
1. SKBR-3 and MDA-MB-468 cells were seeded at 10,000 cells/well and left overnight to adhere
2. pHrodo-labeled antibodies X, Y and Z were added at 300ng/ml
3. Bright field and fluorescent images were captured at 0, 4, 8 and 24 hrs
4. Kinetic internalization can be tracked using Confluence Ratio application on the Celigo

Results:

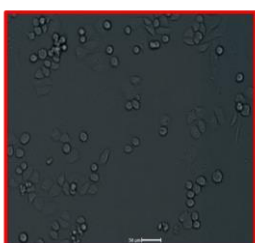
Combined bright field and fluorescent images and numerical data detecting antibody internalization

SKBR3 cells

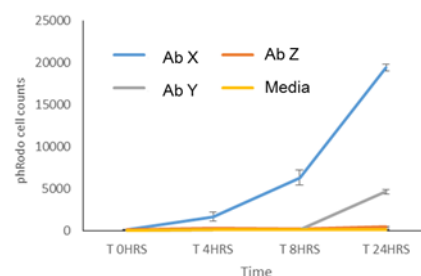
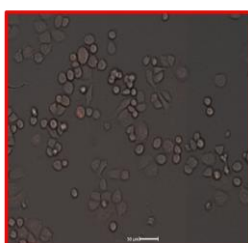
B2: Ab X



C2: Ab Y

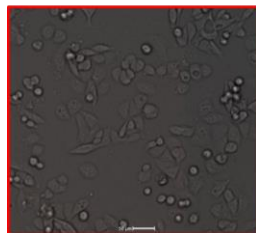


D2: Ab Z

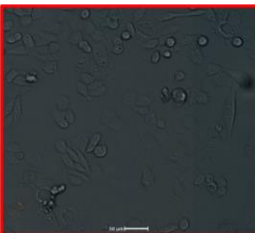


MDA-MB-468 cells

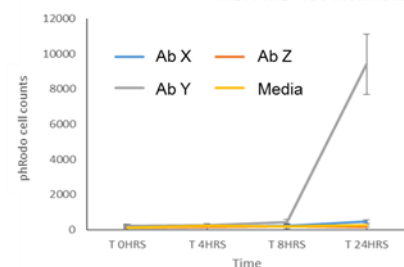
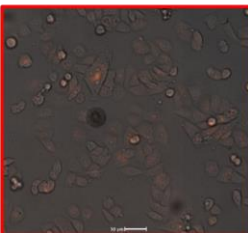
B5: Ab X



C5: Ab Y



D5: Ab Z



- The qualitative pHrodo red fluorescent images overlaid with bright field label free imaging show cellular localization of antibodies using the pH-sensitive dye in real time.
- The quantitative data in the line graphs confirm the previous known activities of antibodies X, Y and Z.

	SKBR3	MDA-MB-468
Ab X	+	-
Ab Y	-	-
Ab Z	+/-	+