

Assay Name: Antibody Cell Surface Binding Detection

Assay ID: Celigo_02_0017

Description: Measure R-PE intensity of cell lines that produce CD3 surface markers

Stains: Hoechst, Secondary Ab tagged with R-PE (Phyco Erythrin)

Imaging channels: Bright field, Red and Blue

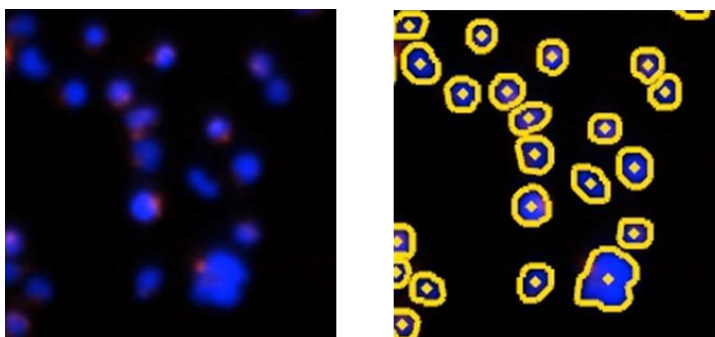
Image analysis algorithm: Celigo software Target 1 + 2 + Mask

Methods:

1. Culture and collect Jurkat and A549 Target cells
2. Seed the Target cells in the wells of 96-well V-bottom microplate containing primary antibody
3. Add secondary antibody tagged with R-PE to the plate
4. Incubate the cells with secondary antibody and Hoechst for an hour. Wash excess stain with PBS ++
5. Transfer the contents to a flat bottom plate to capture images on Celigo. Analyze the total number of cells expressing CD3 cell surface markers

Results:

The Hoechst dye is used to identify cell nuclei and an outline is drawn by the Celigo software where the Red R-PE and blue Hoechst fluorescence are measured



AVG Red integrated intensity from the exported file:

1	0.5	0.25	0.125	0.0625	0.03125	0.015625	0.007813	0.003906	0.001953	0.000977	0
4854.649	5073.738	4699.346	5188.524	4097.635	3705.273	3126.522	3001.83	2501.207	2476.632	2619.501	2305.238
4302.84	4053.183	4395.668	4359.929	3842.659	3485.741	2873.617	2560.465	2387.307	2377.892	2338.967	2420.439
2873.616	2505.686	2281.193	2416.806	2283.204	2456.312	2242.518	2278.264	2040.832	2104.668	2143.343	2086.161
2477.598	2305.663	2364.993	2337.207	2239.795	2632.422	2144.149	2349.716	2211.52	2053.933	2129.419	1971.253
2918.636	2651.051	2316.258	2506.906	2388.193	2540.502	2402.428	2403.611	2517.131	2271.54	2378.676	2241.591
2762.64	2625.949	2408.864	2518.059	2317.881	2500.628	2355.518	2304.565	2306.251	2405.71	2316.755	2325.4
2623.489	2674.788	2418.827	2560.619	2397.319	2587.169	2331.988	2293.822	2444.015	2353.848	2311.957	2399.233
3021.728	2608.374	2607.456	2605.151	2526.204	2646.292	2543.878	2477.816	2532.756	2434.11	3241.211	2203.404

Graph generated by PRISM Graphpad by plotting R-PE Integrated Intensity against Ab concentration:

