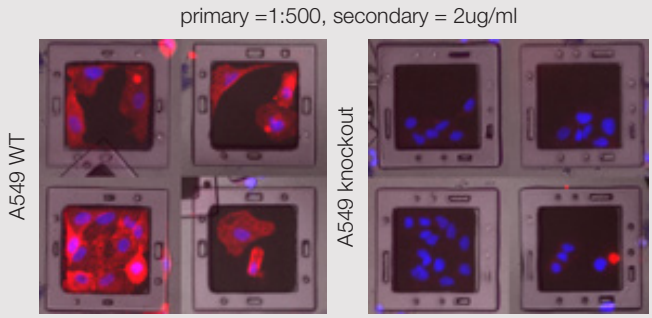
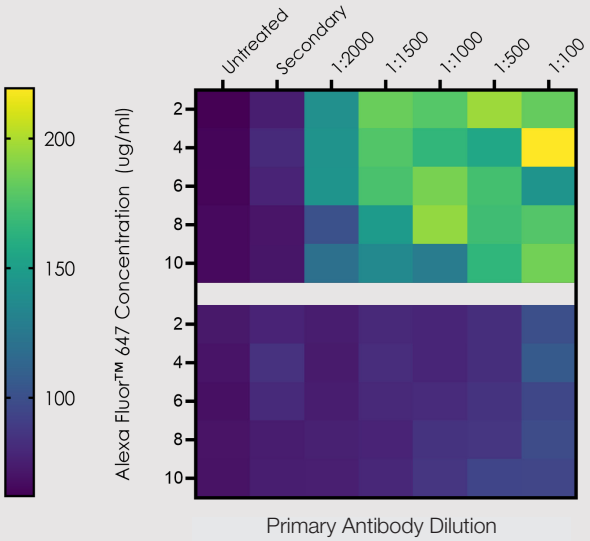
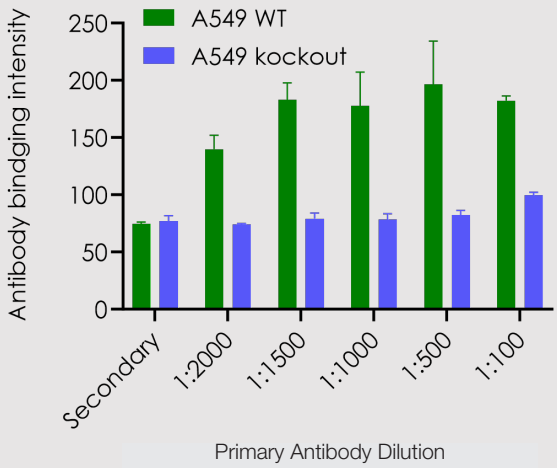
# Antibody Validation Using Design of Experiment (DoE)

- Antibody validation is crucial for assay development, and the gold standard is to perform it across a panel containing both positive and negative cell lines. SemaCytes enable all controls to be housed in a single well, reducing variability and resources needed.
- Multiple factors are important for antibody validation, including primary and secondary antibody concentrations, antibody diluent, wash buffer, and incubation temperature. Therefore, an approach like DoE, which allows for the systematic variation of multiple factors simultaneously, is highly beneficial.
- Given the complexity of assay setup, this approach is well-suited to automation.
- SemaCytes were prepared for both WT and knockout versions of the target of interest. Plates were prepared, and SemaCytes were dispensed, with liquid handling steps performed either manually or with firefly.
- This is a small-scale proof of concept, but with the use of SemaCytes and firefly®, it could easily be scaled up.




| | Total Assay Time (h) | Hands-on Time (h) | Time per Wash Step (min) |
|---|---|---|---|
| **Manual** | 6 | 4.5 | 20 |
| **firefly** | 3.5 | 1 | 3 |
| **Time Saved** | 2.5 | 3.5 | 7 |
| **Multiplex Time Savings** | 5 | 7 | 12 |




## Antibody Validation Using DOE - Workflow Overview

* Seed A549 WT and A549 knockout cells onto SemaCytes with different barcodes
* Leave them overnight and purify.

→

* Mix SemaCytes and dispense into the plate

Note: firefly was not used for this step.

→

* Fix the cells
* Add and incubate with block
* Add primary antibody at the required concentration
* Incubate in the fridge

→

* Wash the cells
* Add secondary antibody and incubate for 1 hour
* Wash
* Add nuclear dye
* Wash

→

* Image the plates
* Perform analysis

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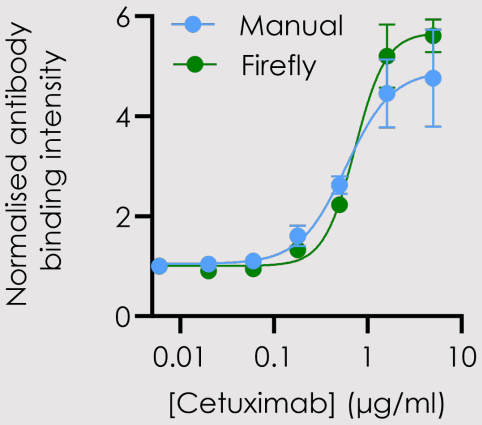
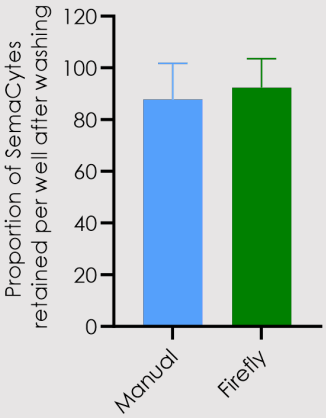
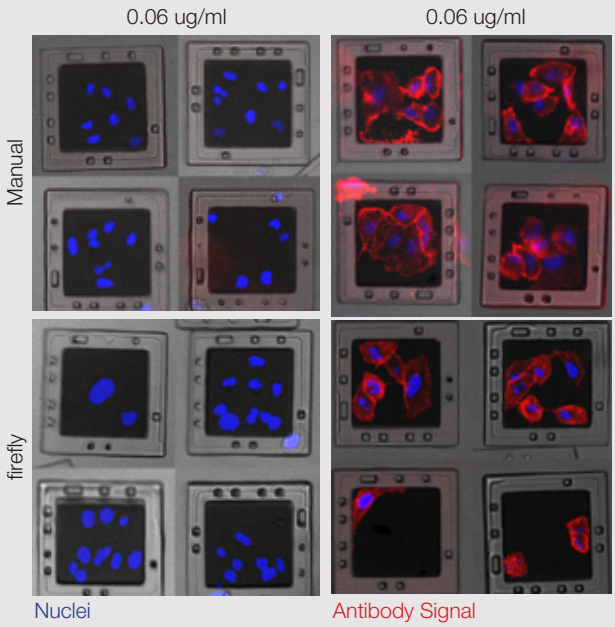
- Typically, this assay is used in antibody discovery, where it is important to understand the binding of candidate antibodies to different variants of the target and related proteins. Currently, these assays are often performed in flow-based assays on suspension cells, but there is a need to explore alternative image-based versions of the assay.
- A549 cells were seeded onto SemaCytes, left for 24 hours to purify, and then dispensed into wells containing primary and secondary antibodies.
- Live cells were incubated for 2 hours at room temperature, washed 5 times, and then imaged.
- Images were tiled using Semalyse, and analysis was performed with FIJI to provide intensity per SemaCyte per well.
- The firefly assay saves time while producing comparable assay quality to manual methods, without the loss of SemaCytes during liquid handling (this data may be highlighted depending on preference).




- 5 washes were performed with the firefly.
- Utilizing firefly for this assay saves 30 minutes of hands-on wash time compared to manually.
- This results in approximately one hour of hands-on time saved per 384-well plate.

## Automated Washing Techniques for Antibody Binding Assays

* Seed A549 cells onto SemaCytes
* Leave them overnight
* Purify

→ * Dispense into the plate

Note: firefly® was not used for this step.

→ * Add primary and secondary antibodies to the wells

Note: firefly® was not used for this step.

→ * Perform five washes

→ * Image the plates
* Perform analysis

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