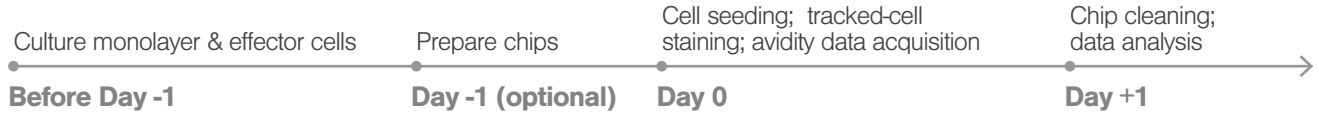


z-Movi[®] Avidity Assay Quick Guide

Version 2

Assay timeline



Essential techniques



Slow flow

With 1.5 mL air cushion, pull to 1.7 mL.



Fast flow

With 0.0 mL air cushion, pull plunger to 3.0 mL.

Pull-through

Using fast flow, pull fluid through the chamber until air flows into the syringe.

Flow-in/Incubate

Flow fluid through the chamber without pulling through air. Leave 10 µL in the reservoir. Uses either slow or fast flow.

Reagent preparation for Day 0

Monolayer cell seeding (all at 37°C)

50 mL serum-free medium + 1.25 mL 1 M HEPES
50 mL complete medium + 1.25 mL 1 M HEPES
1 X PBS
TrypLE (for adherent cells)

Tracked-cell staining & data acquisition

50 mL effector-cell medium + 1.25 mL 1 M HEPES, 37°C
CellTrace Far Red & DMSO
1 X PBS, 37°C

1. Prepare chips

Step 1

Activate with NaOH

1. Incubate 1 M NaOH, 10 min RT
2. Pull through 400 µL dH₂O, 2x
3. Flow in 400 µL dH₂O

Step 2

Coat with PLL or Con A

1. Incubate 100 µL coating, 10 min
2. Flow in 400 µL PBS, 2x
3. Flow in 100 µL serum-free medium
4. Incubate at 37°C until seeding

OR

Rehydrate coated chips

1. Flow in 400 µL 1X PBS
2. Flow in 100 µL serum-free medium

2. Cell Seeding

Step 1

Harvest & resuspend

1. Harvest monolayer cells following [lab protocol](#)
2. Resuspend cells following [cell-line specific protocol](#)

Step 2

Seed cells

1. Flow in 20 µL cell suspension
2. Visually confirm confluency
3. Rinse reservoir, 200 µL medium, 3X

Step 3

Medium refresh

1. First medium refresh at [cell-line specific timing with complete medium](#)
2. Subsequent media refreshes every 2 hours

Dilution formula: mL of medium required for desired concentration: $x = n/y$

n = measured cell concentration (cells/mL x mL of cell suspension)

y = desired cell concentration (cells/mL)

3. Traced-cell staining

Step 1**Prepare stain**

1. Add 20 μL DMSO to Cell Trace vial, mix well
2. For each effector-cell condition, add 1 mL 1 X PBS and 1 μL Cell Trace to 15 mL Falcon tube to create Cell Trace working stock

Step 2**Harvest cells**

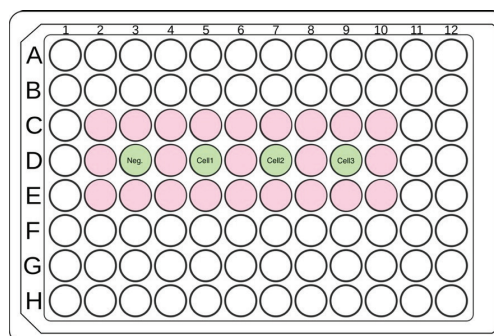
1. Count effector cells
2. Harvest 100,000-200,000 cells, per run, per effector-cell type
3. Dilute effector cells 1:1 with 1 X PBS, centrifuge and aspirate
4. Resuspend in 9 mL 1 X PBS, centrifuge and aspirate

Step 3**Stain, quench, wash**

1. Resuspend in 1 mL Cell Trace working stock per 2 million cells, incubate 15 min in dark
2. Quench in 5x volume complete tracked-cell medium, incubate 5 min at 37°C, centrifuge and aspirate
3. Resuspend in 9 mL complete tracked-cell medium, centrifuge and aspirate.

Step 4**Dilute and plate**

1. Resuspend in complete tracked-cell medium at 10×10^6 cells/mL e.g.: 400,000 require 40 μL medium
2. Plate cells and fill each surrounding well with medium to prevent evaporation
3. Store at 37°C; resuspend each well every 30 min



4. Data acquisition

Oceon™ protocol best practices

- Include a negative control on every chip.
- Run each tracked-cell condition in duplicate, with up to 6 runs per chip.
- Arrange duplicate runs in a different order to control for sequential effects.

Avidity data acquisition

- Follow on screen prompts in Oceon
- Rinse reservoir immediately before using a chip for acquisition
- Screw chip on with screwdriver
- Rinse reservoir during cell incubation, before 1:30 min mark

5. Chip cleaning

Step 1**Remove cells**

1. Empty reservoir
2. Pull through 400 μL dH₂O, 2x
3. Flow in 200 μL Sodium hypochlorite, 5%, incubate 30 min at RT
4. Pull through 400 μL dH₂O, 2x

Step 2**Clean glass**

1. Flow in 100 μL ionic liquid, incubate overnight at RT
2. Pull through 400 μL Tween 20, 2%, 2x
3. Pull through 400 μL dH₂O, 3x

Step 3**Dry chips**

Dry chips completely for storage using a 10 mL syringe and the incubator.

