AeroSense · executed method record · 11 October 2026

B06 · executed pilot

P2 kinetic fluorescence: the executed 96-well experiment

Reproduce the fluorescence time courses and descriptive well metrics reported in Results.

Executed; critical metadata incomplete. Runs 904–935 were acquired on 10–11 October 2026. This is a manual-addition 96-well pilot, separate from the unrun 384-well specification that follows.

Samples, experimental unit and preparation

The team confirmed on 11 October 2026 that HEK293 cells were measured approximately 72 h after transfection, without G418 selection. Only this plate is currently available. The plate contains one well per condition × stimulus; longitudinal readings are technical timepoints, not replicate wells or independent cultures. The labels identify intended construct conditions and do not establish stable clonal lines. Their identity is not automatically matched to the earlier 44–48 h fixed-cell microscopy cultures.

Columns 1–10 are Or7a, Or19a, Or42a, Or98a, Or22a, Or67a, Or67b, Or59b, Or85b and Or35a, respectively, each with ORCO. Column 11 is ORCO only; column 12 is untransfected HEK293. Rows A–H receive VUAA1, ethyl propionate, isopentyl acetate, 3-octanol, benzaldehyde, 2-heptanone, valeric acid and buffer alone, respectively.

The confirmed assay buffer contains 140 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES-Na and 20 mM glucose, at pH 7.3 and 340 mOsm. The team reports initially aqueous VOC preparations subsequently diluted into this buffer. Final VOC concentrations are unknown. VUAA1 uses DMSO; the final DMSO fraction and an equivalent DMSO-only vehicle condition are not documented. The source report lists nominal final VUAA1 at 50 µM, without an independently verified preparation record.

Acquisition and additions

The report identifies a BMG CLARIOstar Plus with SMART Control/MARS and a GREINER 96 F-bottom plate. It records bottom optics, a −4.1 mm focal height, LP 504 dichroic, 20 flashes per well per cycle, 0.5 s settling, enhanced dynamic range off, temperature control off and no shaking during the response read. These are source-reported settings. The instrument CSVs directly confirm the 482–16 excitation / 530–40 emission filter labels and 12 s read spacing; they do not contain numeric gain values.

1. At the reported initial 100 µL volume, acquire F_{0a}: five readings at 0, 12, 24, 36 and 48 s.
2. Add 50 µL plain buffer and acquire the first response, labeled `blank`: fifteen readings from 0 to 168 s, every 12 s. The well now contains 150 µL.
3. Acquire F_{0b} in the same wells: five readings with the same timing.
4. Add 50 µL of the reported 4× working preparation, or buffer for row H, to reach 200 µL. Acquire the `test` response: fifteen readings from 0 to 168 s.

The reported manual sequence uses an eight-channel pipette for columns 1–8 followed by 9–12. Injection timestamps and addition-to-first-read delays are unavailable. Each CSV restarts its time coordinate at its first reading; no interpolation bridges the unmeasured gaps. Header run-start times span 22:11:23 on 10 October to 00:35:25 on 11 October. The final recorded response contains a further 168 s of within-run observations. The headers do not encode a timezone.

Independent calculation

Each F_0 is the mean of five readings. For each 15-reading response, calculate all thirteen contiguous three-reading means and take their maximum, F_{peak3}. This reduces the influence of an isolated point but still selects a maximum; it is a descriptive summary rather than an unbiased effect estimator. The reported peak time is the center of the earliest maximizing window.

> $\Delta F_{buffer} = (F_{peak3,\ buffer} - F_{0a}) / F_{0a}$
> $\Delta F_{test} = (F_{peak3,\ test} - F_{0b}) / F_{0b}$
> $\Delta F_{corr} = \Delta F_{test} - \Delta F_{buffer}$

The two terms use different baselines and well volumes. Their difference is dimensionless; multiplied by 100 it is a difference in percentage points, not a single test-run fluorescence increase. Time-course plots show every measured test point relative to the paired F_{0b}; raw-RFU panels are also provided. No cell-free-background subtraction, gain rescaling, interpolation, fitted response or significance test is applied.

Control-referenced contrasts · added 19 October 2026

Let `R(s,c) = ΔFtest(s,c) − ΔFbuffer(s,c)` denote the existing corrected metric for stimulus row s and cell-condition column c. Figure 14 B reports `R(s,c) − R(H,c)`. Figure 14 C reports `[R(s,c) − R(H,c)] − [R(s,12) − R(H,12)]`. H is a separate buffer-only well of the same cell condition; column 12 is the untransfected well for that stimulus, with H12 as its buffer-only comparator.

These descriptive between-well contrasts use subtraction, not division by a blank. They remain dimensionless differences (×100 gives percentage points), not a new raw ΔF/F0. They do not establish a cell-free background, gain correction or matched DMSO vehicle. All 96 calculations, reference IDs and exclusion flags are preserved in the control-contrast table; B/C retained values stay empty. Ethyl propionate B is omitted from the displayed response maps only, while C stays hatched. H and column-12 algebraic reference zeros are omitted from panels B–C. Panels A–C share a −0.6 to +0.6 scale: red denotes positive values and blue negative values, with no response classification or replicate uncertainty.

Quality control, exclusions and normalization

The source report identifies gain mismatch in both baseline–response pairs of row B and in the first pair of row C. Coherent shifts across wells are consistent with this concern. All 24 corrected metrics from B/C are excluded; C's second-pair arithmetic remains available as exploratory data. The remaining 72 corrected metrics are retained conditionally. Without recorded numeric gain, this does not certify the other pairs as gain-matched. Changing detector gain changes the RFU scale, so comparisons require acquisition consistency rather than a guessed correction from untransfected wells. BMG gain guidance

The independent QC table reports each untransfected stage's first, mean and median RFU, plus the median across the twelve wells of response-mean / paired-baseline-mean ratios. These formulas are explicit. The original QC field named `median_col12_F0a` equals the first reading in each row; its published ratio algorithm is unspecified. The original table is retained for audit, not silently relabeled.

All 96 well summaries retain unmasked arithmetic in clearly labeled `*_computed` columns. The `*_retained` columns leave excluded values empty, never zero. The source's VUAA1 normalization is not used: it includes a negative Or7a denominator and otherwise depends on single, unvalidated reference wells. The recalculated six-well 3 SD value is an audit of the source report only; it is not a limit of detection or a response cutoff. No technical-read error bars, confidence intervals or p values are used to imply biological replication.

Remaining execution metadata

Reproducibility still requires numeric gains, dose and solvent preparation records, matched vehicle wells, seeding density, passage, viability, quantitative detachment, plasmid-batch identity, exact addition delays and measured temperature. The source reports partial detachment, but supplies no measurement that can assign it as the cause of a particular trace. An ionomycin-type reporter control, calcium-dependence test, localization assay and independently repeated stimulation are not part of this dataset. No doxycycline induction is inferred from the published stable-line precedent.

Provenance: supplied P2 report and 32 instrument CSVs; team confirmation on 11 October 2026. This document does not retrospectively apply the separate 384-well specification.

Reproduction files: guide · metadata · analysis script · well summaries.