Fidelta
A Galápagos Company

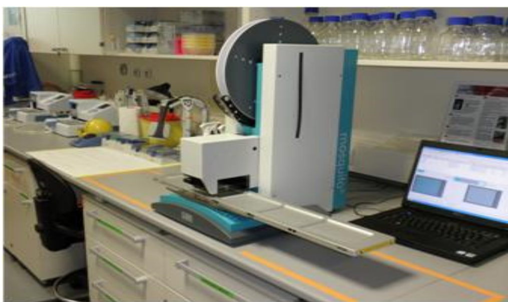
We make the difference in efficacy and safety

www.fidelta.eu

In vitro Pharmacology: Biochemical Screening

In vitro screening for improved discovery and optimization of hits & leads

- More than decade of experience and industry track record in biochemical screening and compound profiling.
- Automatisation and miniaturisation possibilities (utilization of TTP labtech Mosquito nanoliter pipetting platform allows easier setup of the assays - flexible and applicable for low-DMSO-level requirements).
  - ➢ Transfer of as low as 50 nL of DMSO stock solution into a dry plate.
  - ➢ 10 μL assay volume in 384-well plate.



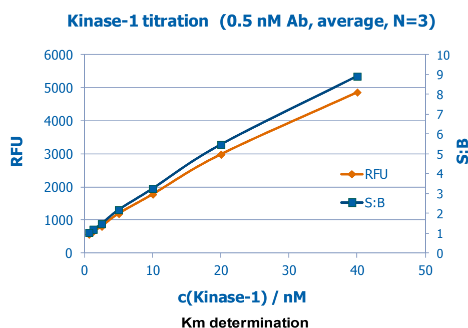
Mosquito nanoliter dispensing unit (X1, TTP labtech) and Multidrops (Thermo scientific).

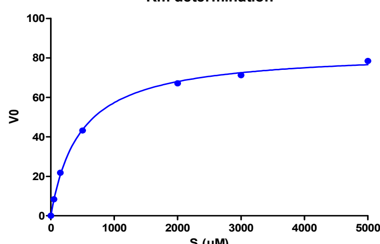
JANUS automatic pipetting workstation and EnVision multimode reader (Perkin Elmer)

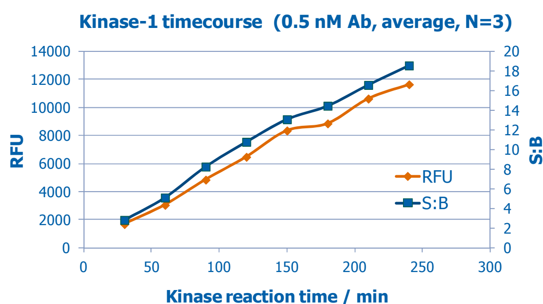
Assay development, optimization and validation

Over 20 years of experience in various *in vitro* compound profiling assays, against vast number of relevant biochemical therapeutic targets in different types of assays:

- Homogeneous assays (in solution) require the addition of all reaction reagents in one well without washing or removal of reagents before reading the plate ('one-pot assay').
- Heterogeneous assays (on a solid phase) involve multiple steps including plate-washing, centrifugation, and aspiration of one reagent and dispensing of another, usually involving more than one incubation step in the process before reading the plate.







- ➢ Primary screening (qualitative)
- ➢ Secondary screening (quantitative)
- ➢ End point assays
- ➢ Functional assays

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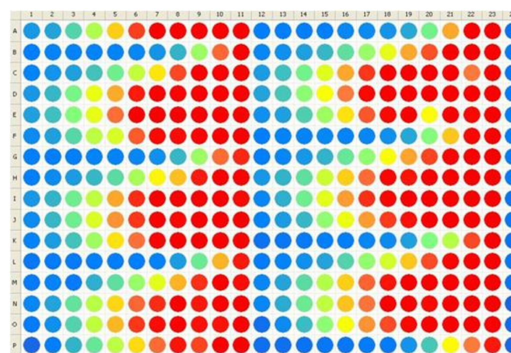
Readout types and screening technologies

The several screening technologies available at Fidelta, assay robustness and performance, together with stringent QC criteria parameters, deliver high-quality research data.

- Absorbance (colorimetry)
- Radioactivity
- Luminescence
  - AlphaScreen (AlphaLISA)
- Fluorescence
  - FLINT (Fluorescence Intensity)
  - TRF (Time-Resolved Fluorescence)
  - FP (Fluorescence Polarization/Anisotrophy)
  - FRET (Resonance energy transfer) – donor/acceptor
  - Cytometry - when the size metrics of cells are assessed by an image processor

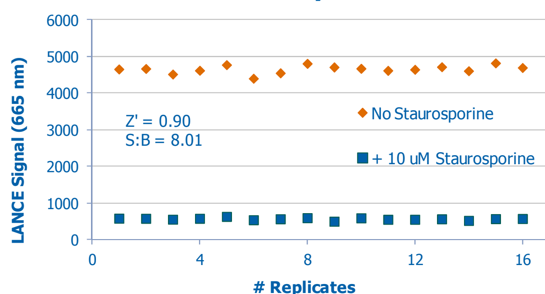


Readout on EnVision multi-mode microplate reader

Assay robustness and performance

- Two series of 16 replicates are analyzed: in the absence (total signal) or presence (minimal signal) of Kinase-1 inhibitor, or in the absence of kinase.
- Target values: %CV of <5 for the total signal, for an S:B ratio from 2 up to >20. These values, combined with a calculated Z'-factor of >0.7, clearly demonstrate the robustness of the optimized assay.

Kinase-1 Assay Precision

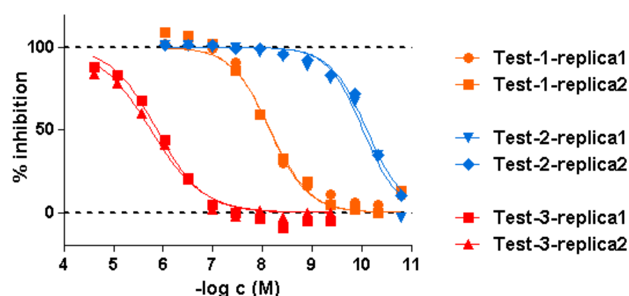


Total Signal		100% Inhibited Signal		Total/100% Inhibited	
Average	%CV	Average	%CV	S/B	Z'
4669	2.31	583	5.26	8.01	0.90

QC (Z', S:B, %CV) analysis for a 384-well LANCE Ultra Kinase-1 assay

Assay results and next steps

Initial screening aimed at hit generation frequently starts with biochemical binding assays and is followed by functional assays.



High-quality assays delivers reproducible data with optimal QC parameters

Data calculation and QC parameters

Calculation of IC_{50} data, curves and QC analysis are done using Excel tools and GraphPadPrism software, v. 5.03. Briefly, individual dose-response curves are generated by plotting the logarithm of the tested compounds concentration (X) vs. corresponding percent inhibition values (Y) using least squares (ordinary) fit. Best fit IC_{50} values are calculated using Log(inhibitor) vs. normalized response - Variable slope equation, where Y=100/(1+10^((LogIC50-X)*HillSlope)).

QC criteria parameters (Z', S:B, R^2, HillSlope) are checked for every IC_{50} curve.

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