

Dianthus uHTS

Tough targets. Confident decisions.

Dianthus™ uHTS is a biophysical instrument for screening of HTS compound libraries. The non-invasive measurement technology allows for direct target engagement screening campaigns and hit validation workflows. Samples are measured in-solution in 1536 well microplates, allowing for rapid speed and near-to-native target conditions.



Figure 1. Dianthus uHTS

Key benefits

Speed. Measure 1536 datapoints fully automated in less than 10 minutes. Perform primary screening of 1.000.000 compounds with a robust Z'-factor in 5 days.

Confidence. Perform direct target engagement studies without prior knowledge of the target's binding site to provide binding data in situations that are not suited to biochemical or FRET approaches.

Versatility. Screen diverse targets regardless of salts, detergents or buffering agents. Determine binding and affinities from millimolar to sub nanomolar concentration for a wide range of ligands.

Automation and manual control

Dianthus uHTS can be integrated into any existing automation systems via a gRPC framework — a high-performance, opensource framework developed by Google that enables remote procedure calls (RPC) between client and server applications — enabling walk away operation and hands-free drug discovery workflow processing. Alternatively, the instrument can be operated as a standalone device via the control software.

Characterization technology

Spectral Shift

Spectral Shift¹ detection quantifies molecular interactions between one binding partner (target) that is labeled with a specific fluorophore optimized to report subtle environmental changes, and another unlabeled binding partner (ligand). Spectral Shift detects ligand induced changes in the hydrophobicity of the target biomolecule's surface by measuring picometer shifts in the fluorescence emission at two wavelengths, 650nm and 670nm. The signal is recorded as ratio between those two fluorescence channels. Hit identification is performed by comparing the signal of the labeled target in the absence of compound in pure buffer (reference) to a reasonably high concentration of the respective compound (ligand). Statistically significant signal changes from the reference (3σ , 5σ , or 7σ) and other quality criteria (e.g. fluorescence signal, scan quality, % of control) are used to distinguish binding from non-binding compounds. Measurements of the labeled target as a function of ligand concentration are used to generate doseresponse curves from which the affinity of the compound can be obtained.

As a rapid, isothermal measurement, it is non-destructive permitting sample re-analysis, temperature-sensitive studies and studies of sensitive molecules that destabilize quickly. The highly sensitive and robust detection allows work with small amounts of labeled target.

System components

The Dianthus uHTS system comprises a benchtop instrument and an embedded control software. A control computer operates the system, and samples are introduced through a plate gate at the front. No fluidics, pumps or valves are required, minimizing daily system maintenance. Dianthus uHTS measures at a stable temperature with active temperature control between 20-25°C.

¹ Langer, A., Bartoschik, T., Cehlar, O., Duhr, S., Baaske, P., & Streicher, W. (2022). A new spectral shift-based method to characterize molecular interactions. *Assay and Drug Development Technologies*, 20(2), 83-94. <https://doi.org/10.1089/adt.2021.133s>

Software

Dianthus uHTS provides a Google Remote Procedure Call (gRPC) interface for integration with laboratory automation scheduling software, allowing seamless connection to other automation platforms and systems. Users can create custom drivers for full automation and customization, tailoring the instrument's functions to specific automation needs and workflows. The Dianthus uHTS control software provides a graphical user interface for manual control of the device. It is embedded in the instrument's computer and is accessible via a browser from any connected PC. Intuitive to operate, it is ideal for manual control of the instrument for assay development and control experiments. Key functions include setting measurement parameters, viewing live experiments, and downloading raw data. The software does not provide extensive analysis capabilities.

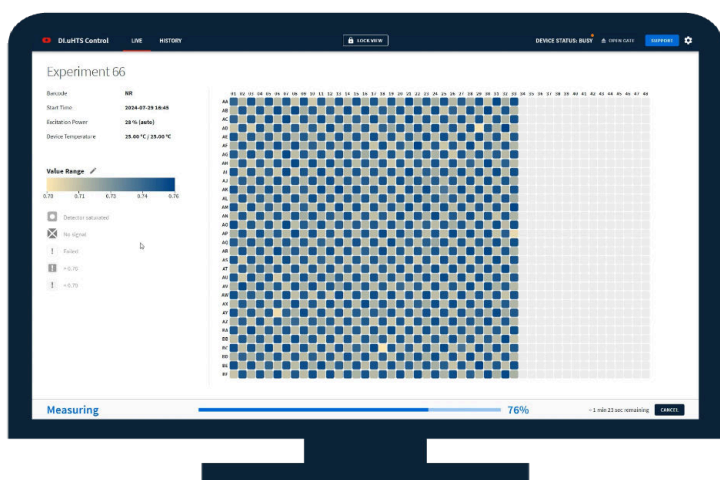


Figure 2. Intuitive experimental set-up with Dianthus uHTS Control.

Data is exported in human-readable .json format, offering simple data integration into enterprise software solutions for data analysis and workflows. System installation and introductory training are performed in just one day.

Additional supporting information is provided online at <https://support.nanotempertech.com/hc/en-us>.



Figure 3. Human-readable JSON export file created by Dianthus uHTS.

Consumables

1536-well microplates

Dianthus uHTS uses dedicated 1536-well, low profile, black walled, sealable, optically clear, flat-bottomed microplates. This plate design enables direct bottom reading of fluorescence signal avoiding well to well crosstalk. Typical assay volume per well is 7 µL. The plates are polymer coated to prevent analyte adsorption and are uniquely barcoded for identification. Custom barcodes are available upon request. The microplates have standard SBS format for compatibility with manual or automated liquid handlers, heat sealing and lidding, as well as automated plate handling devices.

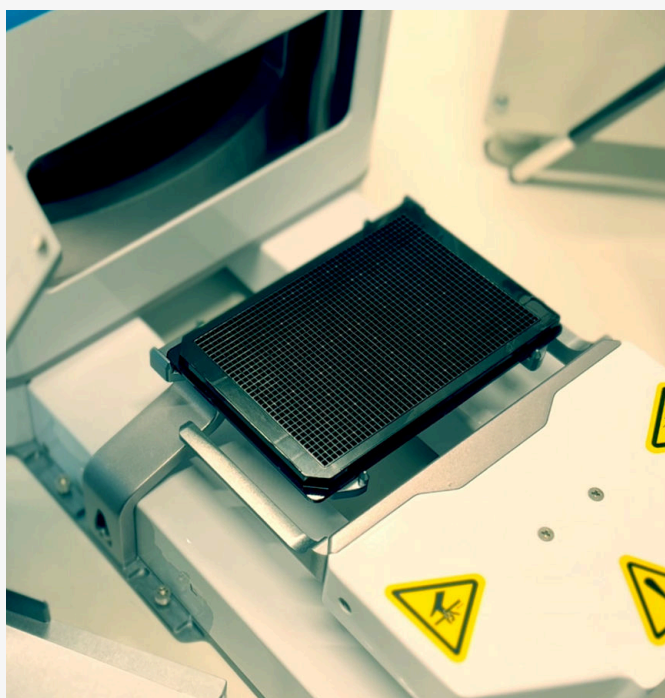


Figure 4. Dianthus 1536-well plate being unloaded from centrifuge.

384-well microplates

Dianthus uHTS enables measurements in standard 384-well microplates. The larger volume of 20 µL per well allows for simple manual operation, useful for assay development and optimization prior to pilot screening and, subsequent, primary screening using Dianthus uHTS 1536-well microplates.

Target Labeling Kits

NanoTemper offers a range of kits specifically optimized for high sensitivity and signal to noise levels. The kits are designed for simple and quick labeling of one binding partner, permitting rapid assay development, optimization and time to data. The kits contain labeling reagents that attach fluorophores to a specific functional group or fusion tag via covalent coupling chemistry or affinity binding. Kit size options are available to accommodate throughput requirements; from small scale assay development to large library screening.

Buffer Exploration Kit

Specifically designed for assay optimization, the buffer exploration kit comprises a plate encompassing 96 selected buffer conditions for easy assessment of target stability and assay windows, simultaneously. The plate can be used for multiple rounds of optimization.

Applications

Dianthus uHTS is a powerful tool for early-stage drug discovery. It provides single point screening data for Hit ID, dose response data for hit verification, and valuable binding (K_d or EC_{50}) information for Structure Activity Relationships (SAR) studies and hit to lead progression.

Dianthus uHTS's in-solution, in-equilibrium measurement enables rapid, non-invasive screening of a wide range of targets and therapeutic modalities, including those typically considered challenging or undruggable. Examples of use cases are shown below. In-depth application data can be found online in the resource center under the Dianthus tab: <https://resources.nanotempertech.com/application-notes>.

1. Primary screening of HTS compound libraries and targeted libraries

Identify hits from HTS libraries comprising up to millions of compounds.

- Routinely generate screening data with Z' scores > 0.5 for provision of assay robustness & confidence in identified hits.
- Measure binding directly for targets lacking functional or biochemical read-outs e.g., transcription factors or scaffold proteins.
- Screen against delicate target classes with strict requirements on buffer composition, e.g., salts or detergents for membrane proteins or nucleic acids, to ensure target functionality.
- Perform direct target engagement irrespective of ligand binding site where FRET based approaches are unsuitable.
- Investigate very low affinity (mM) interactions and assess low Dalton sized compounds, e.g., fragments or ions, problematic for other biophysical methods.

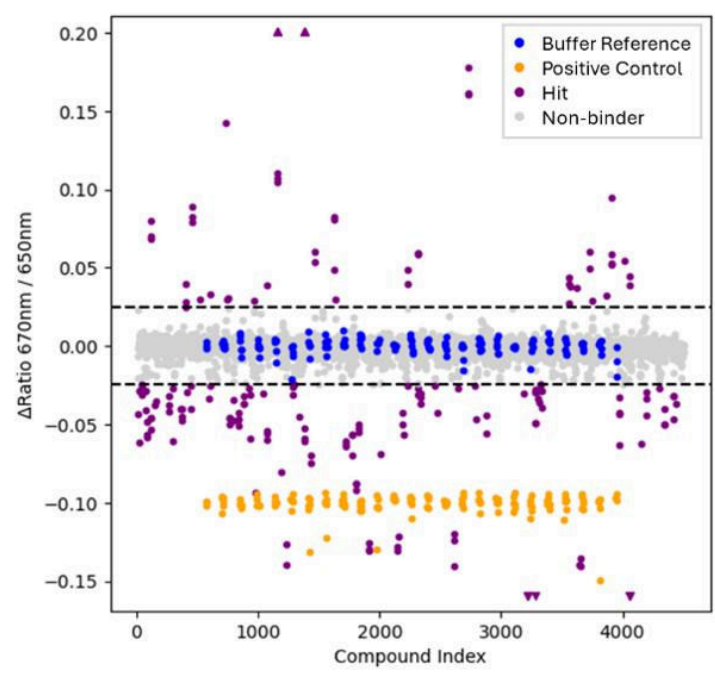


Figure 5. Primary screen of 1280 compounds in triplicates. Hits are identified by significant signal change with respect to the reference and inherent reproducibility.

2. Affinity-based screening and hit validation

Measure dose-response curves for hits from primary screens to validate their interaction with the target. Rank their affinity for triaging and enable progression of screening hits to leads for lead optimization.

- Determine affinities ranging from millimolar to 250 picomolar without changing the assay design.

- Maintain the conformational plasticity of intrinsically disordered proteins (IDPs) by direct in-solution measurement to provide binding studies with data integrity
- Directly measure affinities between nucleic acids and small molecules under the optimal assay conditions, facilitating folding and functionality of the target.
- Study membrane protein interactions when solubilized in detergents or within synthetic membrane models.

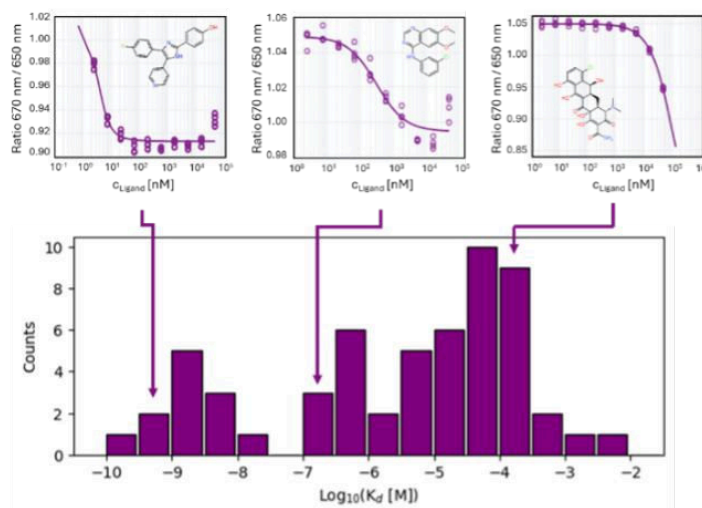


Figure 6. Affinity histogram of 57 compounds. Primary hits were confirmed by measuring 10-point dose-response curves in triplicate. Dose responses with K_d -fit are shown for selected examples of a subnanomolar target-selective kinase inhibitor (A), a non-selective kinase inhibitor (B), a weak binding compound with affinity > 100 μ M (C), and a non-binding compound (D). The dashed line represents the baseline ratio of unbound target from the primary screen.

3. Additional applications - Research and Development

The in-solution nature of the Spectral Shift technology allows for a variety of additional assays.

- Record time-resolved measurements of covalent binding interactions to derive reaction kinetics in high throughput.
- Analyze modulators of protein-protein interactions, including degraders and molecular glues, for complex affinities, cooperativity, and hook effects, as well as study inhibitors that disrupt protein-protein or protein-DNA complexes.
- Setup simple displacement assays with fluorescently labelled tracer molecules to obtain insights into binding modes of the ligands.
- Design direct-target engagement assays for nucleic acids, including RNAs, with simple assay design.

Specifications

General specifications

Detection technology	Spectral Shift
Information obtained	Intensity information from two fluorescence channels (FI 650 nm, FI 670 nm), ratio, well scan, well scan quality
Single dose data	Binder, non-binder, outlier, auto-fluorescence, quenching, aggregation (analysis in third party software)
Dose response data	Dose-response: K_d , EC_{50} (analysis in third party software)
Outlier data	Aggregation, saturation, quenching, autofluorescence
Data Presentation	Interactive Plate Heat Map Graphic
Data export format	.json export files
Sample types	Small molecules, fragments, ions, heterobifunctional degraders ² , peptides, proteins ³ , biologics, DNA/RNA
Sample Volume	7 μ L / well (1536-well plate), or 20 μ L / well (384-well plate)
Sample format	Black, flat clear bottom, sealable, 1536-well barcoded plates, 6 - 9 μ L working volume or black, flat clear bottom, sealable, 384-well barcoded plates, 18 - 25 μ L working volume
Sample capacity	One 1536-well plate or one 384-well plate per run
Affinity range	250 pM to mM
Data points per run	1536 / 384 data points per run
Run time	< 7 minutes for full 1536-well plate, ~ 30 minutes for 384-well plate
Time to obtain a K_d (12-point dilution series)	< 3.3 seconds when measuring a full 1536 well plate, < 60 seconds when measuring a full 384 well plate
Reproducibility of affinity measurement (32 biological replicates)	Mean EC_{50} = 29 μ M 95% CI [23.1 μ M to 36.6 μ M]
Molecular weight range	10 ¹ - 10 ⁷ Da
Automation	Able to integrate with liquid handlers, robotics & automation platforms via gRPC framework

Electrical

Input voltage	Single phase; AC 100-240 V -10 % +10 %
Mains frequency	50/60 Hz
Input current	AC 6 - 3.2 A
Fuse	Fuse link 5 x 20 mm, 10 A, 250 V, time-lag T (2x)

² Existing Dianthus users can contact us to explore upgrade options to Dianthus α .

³ Including but not limited to intrinsically disordered proteins, membrane proteins, enzymes, and transcription factors

Computer requirements

Operating system	Windows 10 64 bit or higher, English language
CPU	12 th Gen Intel Core i5 or better
RAM	≥8 GB
Hard drive	≥60 GB free disk space
Display resolution	1920 x 1080 or better

Software	Microsoft.NET 4.7.0 & Microsoft.NET Core 3.1
Network	1000 Mbps Ethernet connection

Environmental, Temperature, and Dimensions

Size	Width: 61cm (24.0") Height: 42cm (16.5") Depth (closed tray): 57cm (22.4") Depth (open tray): 69cm (27.2")
Weight	70kg (154.3 lbs) net
Operating temperature	20 – 30 °C (indoor only)
Humidity	5 – 70 %, non-condensing
Temperature control range	20 – 25 °C
Max difference to room temperature	± 5 °C
Precision of temperature control	± 10 °C
Pollution Degree	2


Ordering information

Product	Code
Dianthus uHTS <i>incl Control PC, Dianthus uHTS Software package and automation API</i>	DI-013001
Dianthus 384-Microwell plates	DI-P001
Dianthus uHTS 1536-Microwell plates	DI-P002
Spectral Shift Optimized Protein Labeling Kit — Biotin-Reactive	XI: NT-L220
Spectral Shift Optimized Protein Labeling Kit — Lysine-Reactive	L: NT-L121 XI: NT-L221
Spectral Shift Optimized Protein Labeling Kit — Cysteine-Reactive	L: NT-L124 XI: NT-L224
Spectral Shift Optimized Protein Labeling Kit — For His-Tag	L: NT-L128 XI: NT-L228
Labeling Kit RED-NHS 2nd Generation	L: NT-L111
Labeling Kit RED-MALEIMIDE 2nd Generation	L: NT-L114
His-Tag Labeling Kit RED-tris-NTA 2nd Generation	L: NT-L118
Biotinylated Target Labeling Kit	L: NT-L120
Human Fc Labeling Kit	L: NT-L130
Buffer Exploration Kit (96 plates)	NT-B001

Note: all NanoTemper labeling kits are compatible with the Monolith X, Dianthus, and Dianthus uHTS.

Compliance

Safety	IEC 61010-1:2010/AMD1:2016 Part 1 IEC 60825-1:2014 21 CFR 1040.10 and 1040.11 except for conformance with IEC 60825-1 Ed. 3., as described in Laser Notice No. 56, dated May 8, 2019 EMC IEC 61326-1:2021
Overvoltage Category	CAT II
Laser Classification	Laser Product Class I



Scan the QR code to open the Dianthus uHTS product page.
nanotempertech.com/dianthus-uhts
 For local office contact information, visit
nanotempertech.com/offices