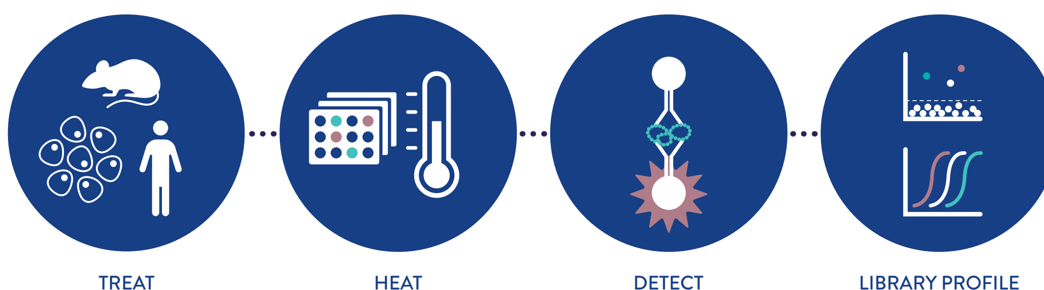
PELAGO
BIOSCIENCE

Is your hit on target?

Lack of efficacy due to poor target engagement is a major reason compounds fail during phase II clinical trials. High-throughput screening (HTS) is widely used in small molecule drug discovery to identify chemical starting points on novel therapeutic targets. The evaluation of hits and the subsequent development into lead molecules by use of structure-activity relationship (SAR) is a time and effort consuming task. Confirmation of hits and consequent prioritisation enables efficient generation of lead molecules.

The patented Cellular Thermal Shift Assay, CETSA® can determine target engagement in physiologically relevant settings and therefore reduce the failure rates and associated high costs of drug discovery programs. The adaptation of CETSA into a microtiter plate format enables compound screening with high-throughput: CETSA HT, with applications in hit identification, confirmation and lead generation.



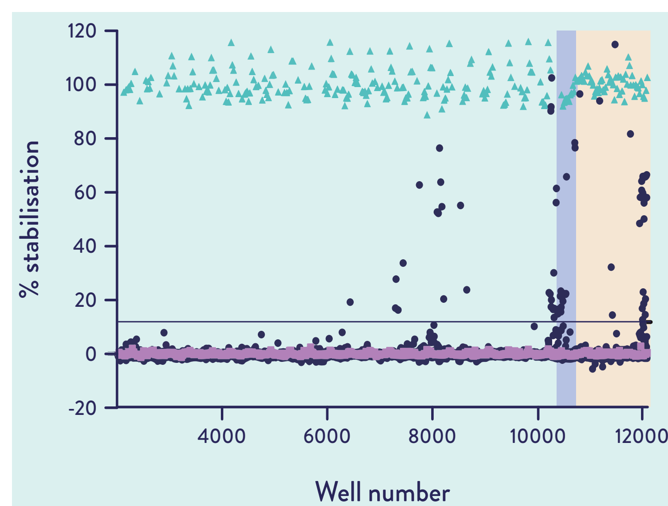
CETSA HT can evaluate target engagement in the relevant physiological environment

CETSA HT efficiently assesses target engagement in live cells, on endogenously expressed target in a label free system. In the high throughput mode (384 well), CETSA HT can evaluate large compound libraries and multiple conditions with a short turnover (N=2 in one week). The homogenous dual antibody detection technology, allows rapid and sensitive quantification with no need to separate folded from denatured proteins in the sample[1]. Target levels can be quantified by dual antibody proximity detection systems based on e.g. chemiluminescence (AlphaLISA®) or fluorescence (Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET), that can be read on standard multimode plate readers.

LIBRARY SCREENING

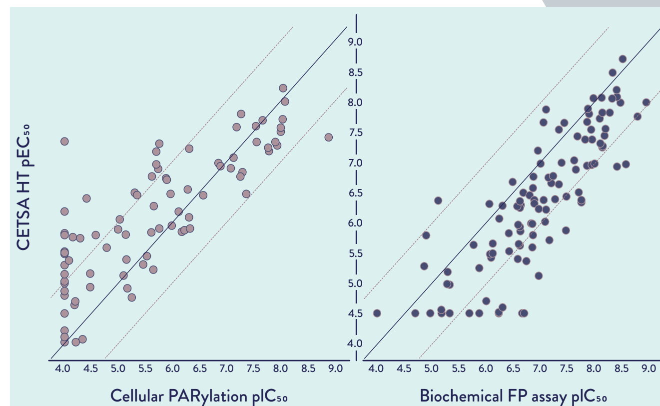
Example of library screening identifying novel hits of thymidylate synthase (TS) inhibitors with cellular activity, including compounds that require metabolic activation before interaction with TS[2].

CETSA HT library screening of >10,000 diverse compounds. The scatter plot shows compound thermal stabilisation (blue circle) normalised to 100% stabilisation by 100 nM raltitrexed (green triangle) and 0% control with DMSO only (magenta square). Black line = hit limit. Purple area: nucleoside analogues. Orange area: Prestwick library of annotated reference compounds and marketed drugs.



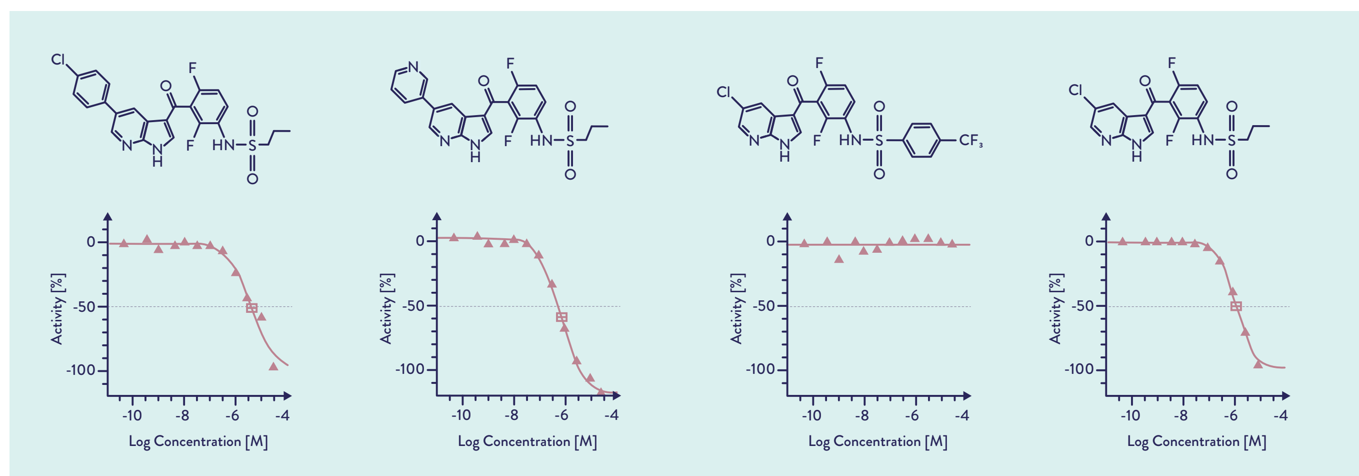
HIT CONFIRMATION

CETSA HT was applied for hit confirmation following a HTS campaign against a clinically validated target (PARP1) based on a biochemical fluorescent polarization (FP) assay[3]. In summary, the activity of the most potent PARP1 binders were confirmed in CETSA HT, with the advantage of instant confirmation of their permeability and target engagement in the cell. Interestingly, potencies were comparable between the CETSA HT and the cellular assay while higher potencies were observed in the FP biochemical assay illustrating the differences between biochemical and cellular assays.



EC_{50} and SAR

To identify B-Raf inhibitors, the CETSA HT assay was adopted to screen a focused library of 896 kinase inhibitors. 13 hits were identified including well-described B-Raf inhibitors and other structurally related compounds[3]. CETSA HT EC_{50} and ranking information can be utilised for SAR analysis in lead generation and optimisation.



As drug discovery strives to become more efficient at delivering successful clinical drug candidates, cell-based assays need to better reflect the targeted disease. One key advantage of CETSA HT is the potential to confirm that compound mediated cellular effects are a consequence of target engagement in cells. CETSA HT can be applied to various stages of drug discovery from hit identification to candidate drug selection.

References:

1. Jafari et al. Nature Protocols 2014
2. Almqvist et al. Nature Communications 2015
3. Shaw et al. SLAS Discovery 2018

Figures in this application note are modified from original.