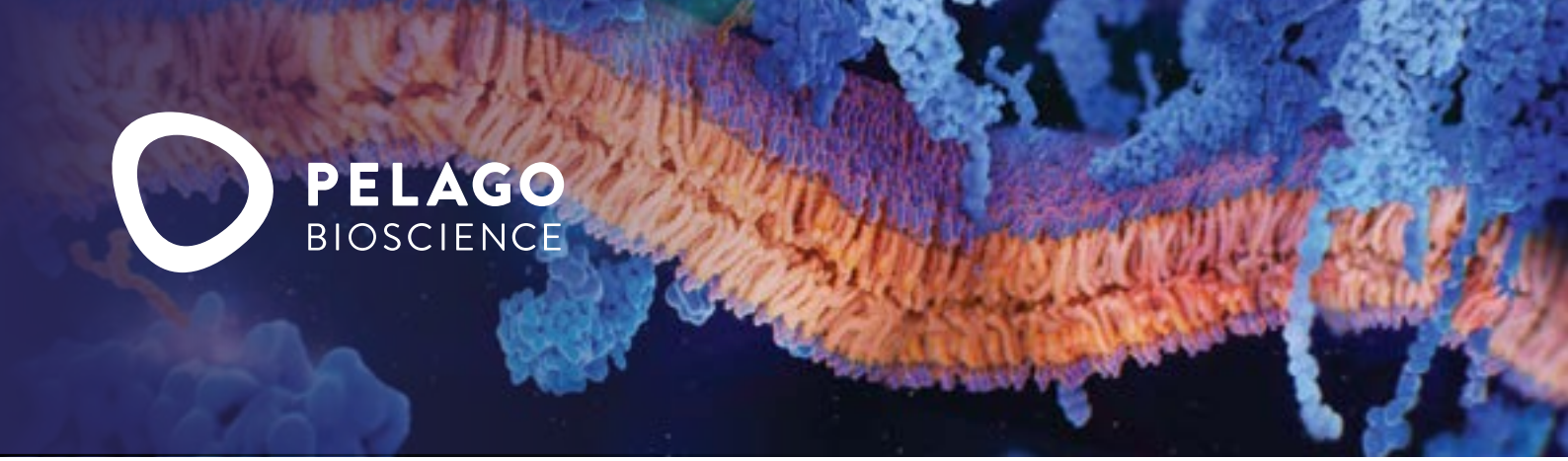
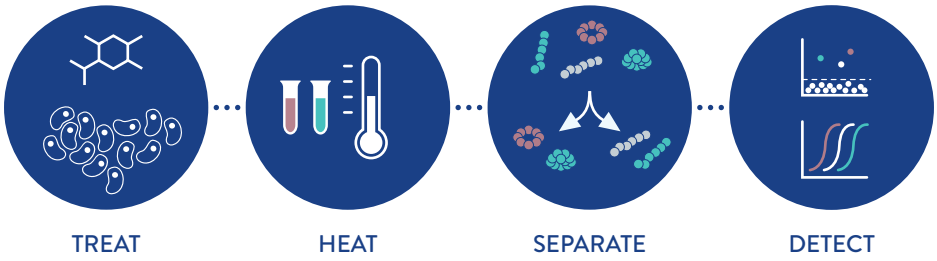


PELAGO BIOSCIENCE

Is your hit on target?

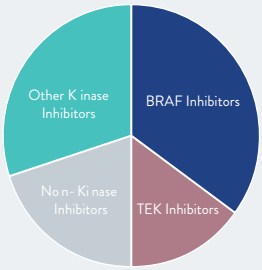
Lack of efficacy due to poor target engagement is a major reason compounds fail during phase II clinical trials. High throughput screening (HTS) is widely used in small molecule drug discovery to identify chemical starting points on novel therapeutic targets. The evaluation of hits and the subsequent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



CETSA® Navigate HT can evaluate target engagement in the relevant physiological environment

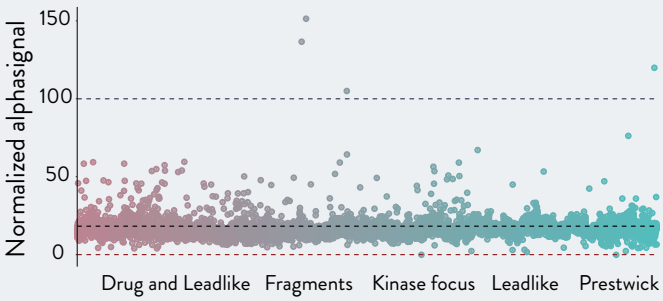
CETSA® Navigate HT efficiently assesses target engagement in live cells in a high throughput mode. With CETSA® Navigate HT it is possible to screen large compound libraries and test multiple conditions with a short turnover. The homogenous dual antibody detection technology, allows rapid and sensitive 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.

CETSA® Library Screening identifies relevant chemistry



In a recently published study by AstraZeneca, 0.5 million compounds were screened against protein kinase raf 1, CRAF using the CETSA® Navigate technology.[2] The screen identified both known inhibitors of CRAF and novel chemistry and the hit compounds could broadly be categorised into 5 groups.

For a large group, activity had already been demonstrated for the related kinase BRAF, suggesting that the CETSA® Navigate HT assay was capable of identify hits expected to have potential for activity against CRAF. The study proved the assay to be robust for screening and with a very low number of false positives.



Pelago screened a 11,000-compound library which included lead like compounds, a focused library as well as Prestwick and Fragment libraries, against cyclin dependent kinase 4, CDK4.

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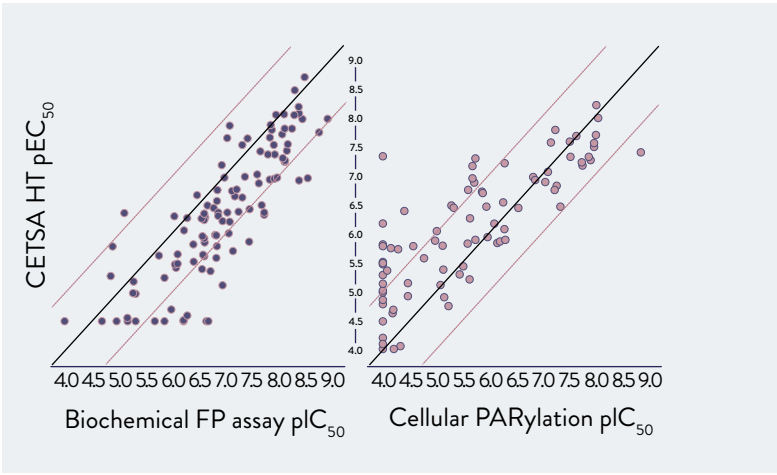
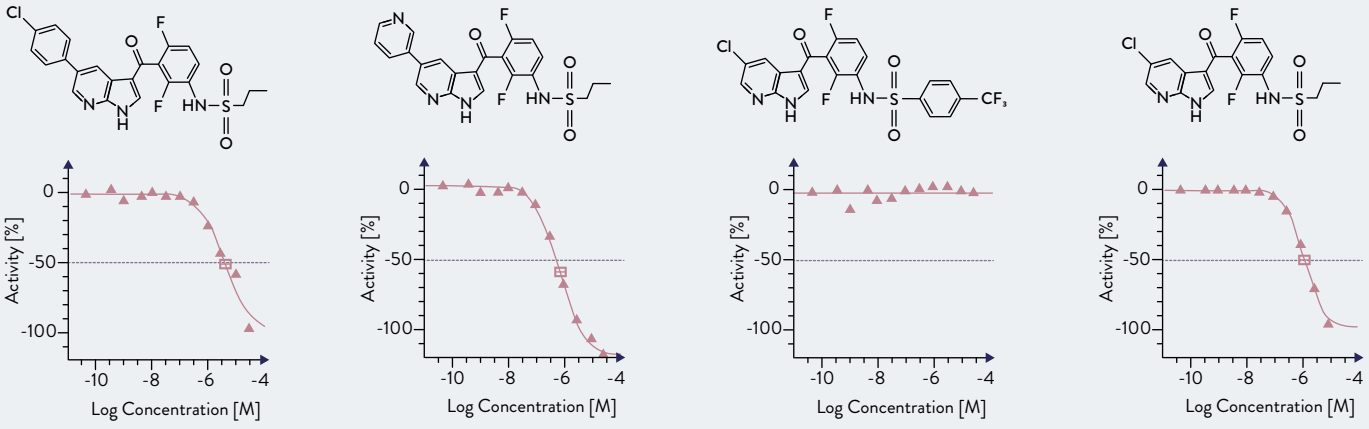
Hit confirmation

CETSA® Navigate HT was applied for the hit confirmation against a clinically validated target (PARP1). CETSA® EC_{50} values were compared against previous PARP1 HTS campaigns at AstraZeneca: a biochemical fluorescent polarization (FP) assay and a cellular PARylation assay.[3] The activity of the most potent PARP1 binders were confirmed in CETSA® Navigate HT, with the advantage of instant confirmation of their permeability and target engagement in the cell. Interestingly, potencies were comparable between the CETSA® EC_{50} and the cellular assay, and a number of identified CETSA® hits (that bind the target) do not translate to functional inhibition of PARP1 (silent binders). Utilising CETSA® Navigate HT and by correlating with orthogonal assays we gain a deeper understanding of the compound effect in a complex model.



EC_{50} and SAR

To identify B-Raf inhibitors, the CETSA® Navigate 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® Navigate 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® Navigate HT is the potential to confirm that compound mediated cellular effects are a consequence of target engagement in cells. CETSA® Navigate 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. Rowlands et al. SLAS Discovery 2023
3. Shaw et al. SLAS Discovery 2018

Figures in this application note are modified from original.