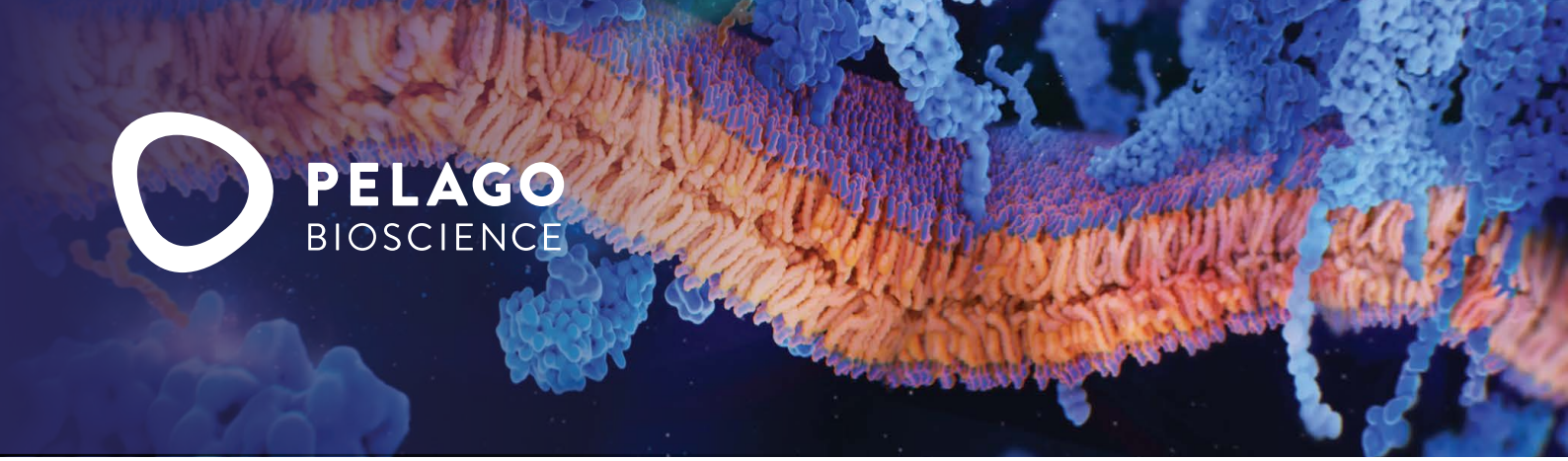


PELAGO BIOSCIENCE

Is your target difficult to drug?

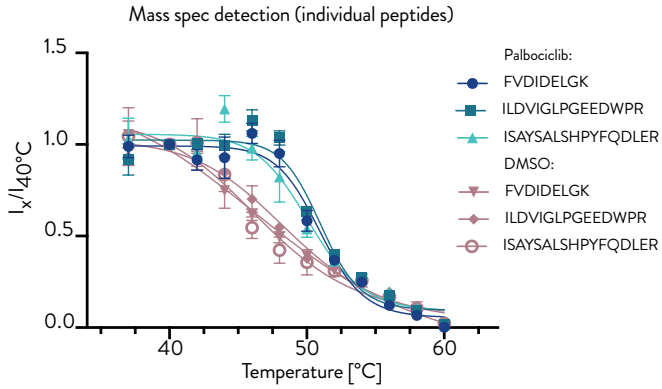
Developing therapeutics against novel and difficult-to-drug targets is one of the key challenges of modern drug discovery and development[1]. One of the main challenges with unexplored novel protein targets is often the lack of tools, such as antibodies, for the investigation and design of compounds that interact with these protein targets.

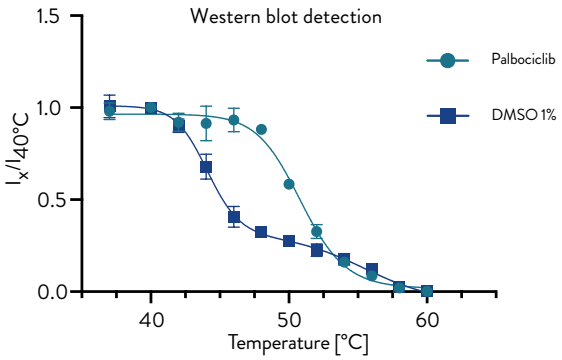
The Cellular Thermal Shift Assay (CETSA®) is a well-established, proven, and accurate technique for measuring compound binding to target proteins[2]. The CETSA® method is based on the principle that a protein bound to a ligand has different thermal stability than the unbound protein.

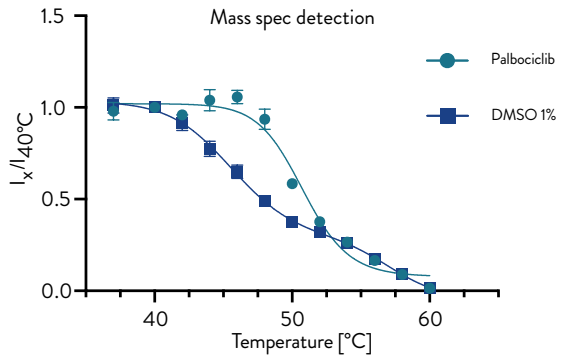
CETSA® Navigate MS has been developed to assess the target engagement of compounds towards novel targets using MS readout reliably and effectively. Instead of relying on the access of detection antibodies, CETSA® Navigate MS uses trace peptides as internal standards allowing for specific and robust profiling of individual or multiple proteins in parallel. Therefore, using this method, it is also possible to multiplex and study several proteins at the same time. CETSA® Navigate MS is a valuable method to investigate different isoforms or to perform selectivity studies and to assess target engagement early in your drug discovery project.

Targeted CETSA® Navigate MS can be used to assess target engagement against novel protein targets

In a proof-of-concept study of the CETSA® Navigate MS, we focused on cyclin-dependent kinase 6, CDK6, a well-established onco-target protein[3]. A previously established CDK6 CETSA® Navigate assay, based on western blot detection, was used for benchmarking of the CETSA® Navigate MS assay. Pelago Bioscience profiled the cellular target engagement of CDK6 by the kinase inhibitor Palbociclib (Ibrance®) in K562 cells using the two formats in parallel to demonstrate that both assays reliably and efficiently can be used for evaluating cellular target engagement.







During assay development, the CETSA® melt curves are generated by heating the compound treated sample and the vehicle control (DMSO) in ascending order. When the compound-induced shift is characterised, a fixed temperature is used to establish the concentration-response curve (50°C in this example)[2].

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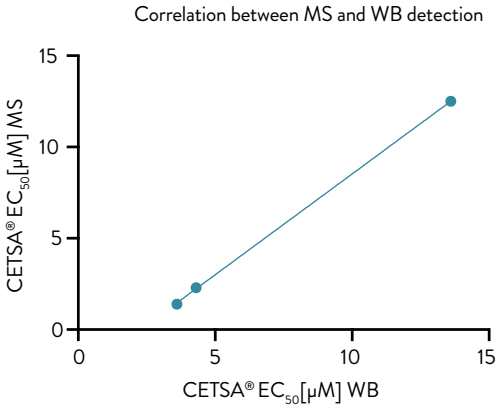
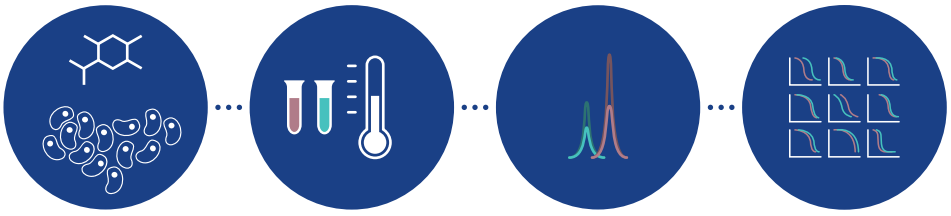
Correlation of CETSA® EC_{50} values between the CETSA® Navigate assays using Western Blot and Mass Spec detection

To determine the CETSA® EC_{50} potencies, cells are incubated with increasing compound concentration and heated to a fixed temperature (50°C in this experiment). This generates a concentration-response at a specific temperature which can be used for potency ranking of compounds. In this example, cells were incubated with Palbociclib, Ribociclib, and Abemaciclib to generate CETSA® EC_{50} for the three different compounds.



Compound	CETSA® EC_{50} [µM] WB	CETSA® EC_{50} [µM] MS
Palbociclib	4.3	2.3
Ribociclib	13.6	12.5
Abemaciclib	3.6	1.4



CETSA® Navigate MS provides you with the possibility to study previously un-druggable targets that lack tools such as detection antibodies. It allows for the efficient verification and validation of target engagement of your compound, in the native physiological environment. CETSA® Navigate MS can be used to study different isoforms or to performv selectivity studies. It also allows for multiplexing, making it possible to study several targets at the same time.

References

1. Soucek et al. Nat Rev Cancer 2017
2. Martinez Molina et al. Science 2013
3. Sexl et al. Int J Cancer 2020

Figures in this application note are modified from original.