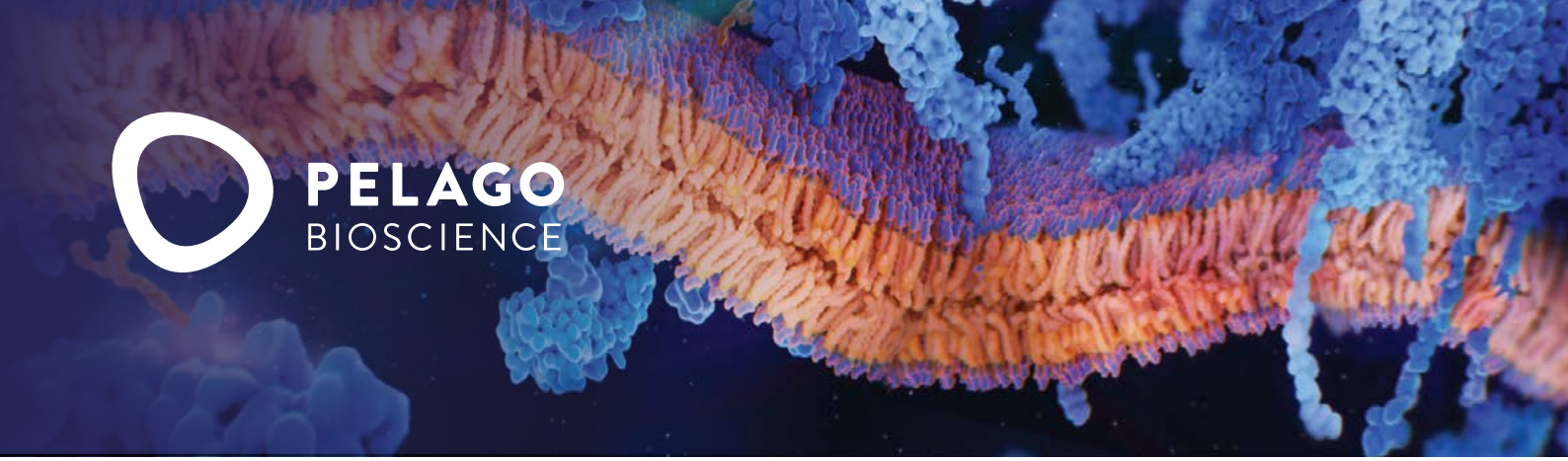
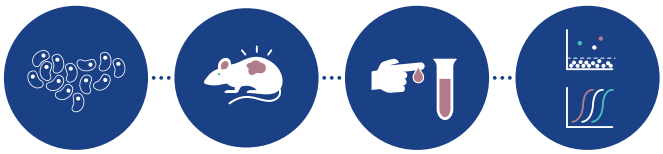


PELAGO BIOSCIENCE

Is your compound actually therapeutic?

It's essential for a successful drug discovery campaign that your compound is therapeutic, it must selectively bind the right target in order to be both effective and safe. To achieve this, the drug must be present at the site of action and occupy the intended target with high specificity. Most drug candidates fail in the proof of concept stage due to inadequate efficacy, which often can be attributed to insufficient occupancy or the lack of target engagement.

The patented Cellular Thermal Shift Assay (CETSA®) enables ranking of compounds by their ability to engage the target in a physiologically relevant setting. Confirmation of target engagement allows you to proceed with the right candidate from the start. CETSA® easily transfers from cell line to animal tissue and human primary cells. Conventional affinity assays measuring a functional outcome or downstream pharmacodynamic effect are not reliable for a large number of potential drug targets, due to poor sensitivity, specificity or artefacts. By measuring a shift in the target's melting temperature induced by binding of the ligand within the cell, CETSA® quantifies the potency of target engagement across a plethora of sample types and species, indicating whether or not your drug candidate will have a therapeutic effect in patients.

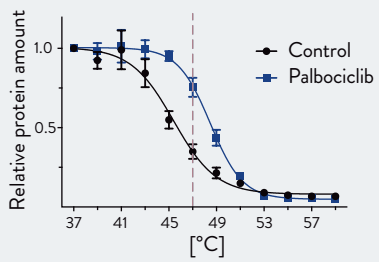


CETSA® transfers from tumour cell line to xenograft

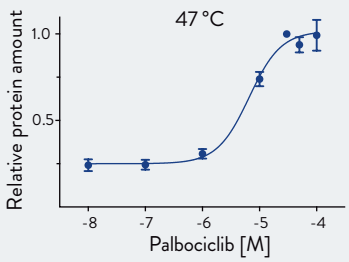
Pelago Bioscience analysed the target engagement of the cyclin dependent kinase 4 (CDK4) by the kinase inhibitor Palbociclib (Ibrance®) in a tumour cell line and subsequently translated the target engagement assay into human xenograft. CDK4/6 inhibitors have substantial anti-proliferative activity in patients with advanced hormone receptor-positive (HR+) and human epidermal growth factor receptor 2-negative (HER2-) breast cancer.[1]

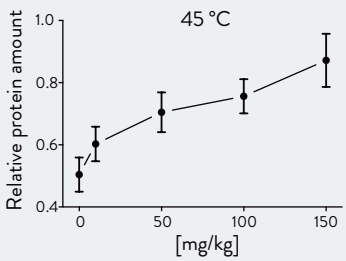
Once established, the CETSA profile of CDK4 in presence and absence of the CDK4/6 inhibitor Palbociclib target engagement is explored in tumor xenografts from Palbociclib dosed mice.

A.



B.





In vitro

A. Establishment of the CETSA® melt profile of CDK4 with inhibitor Palbociclib (blue) or control (black) in intact cells.

B. Corresponding concentration response curve to determine target engagement potency at a fixed temperature.

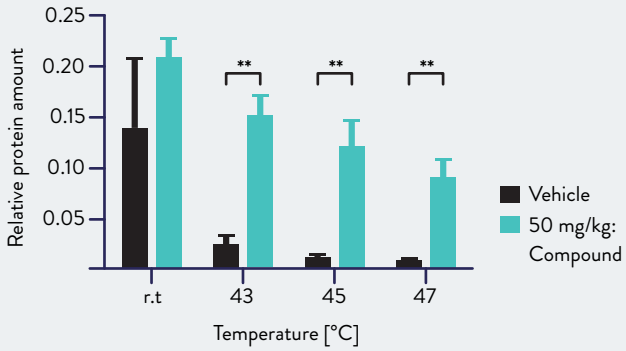
In vivo

Xenograft melt and shift curves show a concentration dependent increase in target engagement in cancerous tissue.

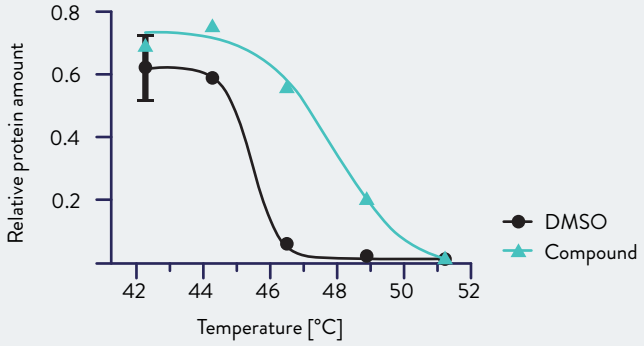
PELAGO BIOSCIENCE

CETSA® transfers from mouse brain tissue into human primary cells

CETSA® enabled evaluation of selective inhibitors of the enzyme RIPK1 (Receptor interacting protein kinase 1). Based on the CETSA® results, a compound was selected for further studies in a mouse model of multiple sclerosis (MS).[2]



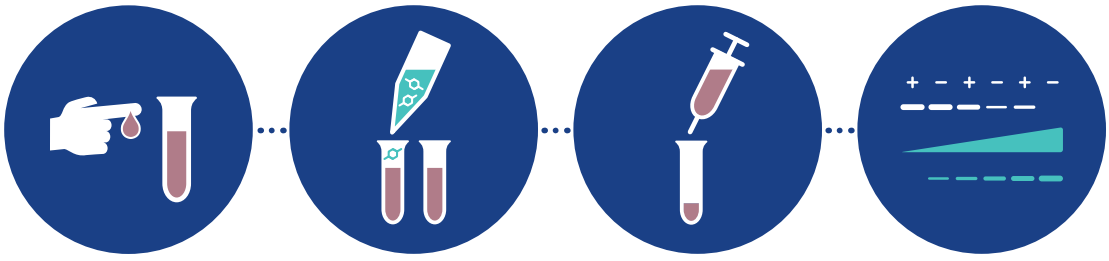
CETSA® in brain tissue
Orally administered 50 mg/kg of compound or vehicle control. One hour after administration, brain samples were collected and divided into four parts, individually heated at room temperature (r.t.), 43, 45 and 47 °C. Based on Western blot data, thermal stability is estimated from chemiluminescent intensity. ** - significant at $p < 0.01$.



***Ex vivo* human PBMC assay**
Human PBMCs were heated at a range of temperatures for 8 min in the presence of 1 µM compound or DMSO. The target displayed increased melting temperature, demonstrating ex vivo target engagement by the compound.

After compound administration, the target protein showed a significant increase in thermal stability indicating that the unbound compound concentration in the brain was sufficient to show *in vivo* inhibition of mouse endogenous RIPK1.

Once demonstrated that the compound engaged the target in the relevant tissue, human peripheral blood mononuclear cell (PBMCs) were selected as a human reference to investigate RIPK1 target engagement in primary cells.



CETSA® offers an artefact free measurement of target engagement to help you make informed decisions about your compound faster, with an assay that can be easily translated into relevant tissue and human samples.

References:

1. Taylor-Stocke et al. Breast 2019
2. Ishii et al. Nature Scientific Reports 2017

Figures in this application note are modified from original.