PELAGO BIOSCIENCE
The home of CETSA®

NO TARGET, NO THERAPY— TARGET ENGAGEMENT WITH CETSA®

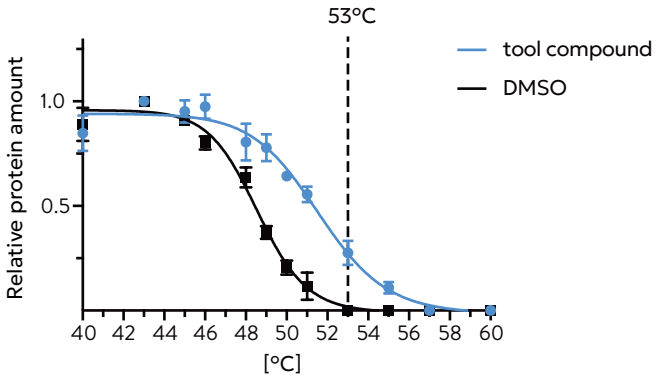
CETSA® delivers direct, physiologically relevant target engagement data to rank and prioritize drug candidates early

A drug's efficacy depends on its binding to the intended target and eliciting a functional response. The relationship between target engagement and efficacy is crucial, as insufficient target engagement is a significant reason many drug candidates fail to progress to Phase III clinical trials. Potency ranking with Pelago Bioscience's CETSA determines the potency of compounds directly in cells or tissues of interest, providing critical target engagement data independent of function—early in the drug discovery pipeline.

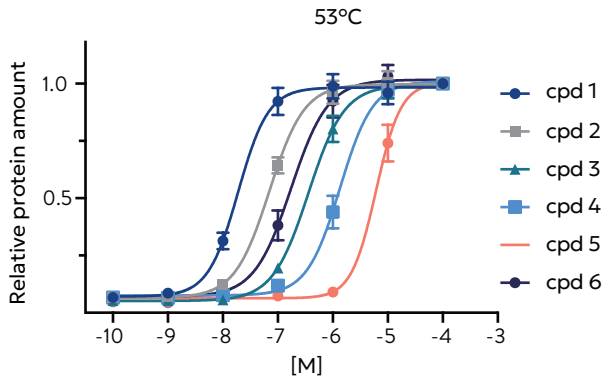
CETSA can be used to verify and validate target engagement

CETSA EC_{50} provides valuable insights into your compound's potency. As it is a relative measure, testing at least three compounds at a fixed temperature is recommended. This approach allows for ranking and prioritization of lead compounds. The CETSA derived EC_{50} values depend on the cellular environment and assay parameters, ensuring physiologically relevant insights.

Figure 1 displays the key experimental phases in determining EC_{50} potencies.



Step 1 – During assay development, CETSA melt curves are generated by heating compound-treated samples and vehicle controls (DMSO) in increasing temperatures. Once the compound-induced shift is characterized, a fixed temperature is selected for concentration-response studies (53°C in this example)1,2.



Step 2 – To determine CETSA EC_{50} potencies, cells are incubated with increasing compound concentrations and heated to a fixed temperature (53°C in this experiment). This establishes a concentration-response relationship between the target protein and the compound. Compound 1 exhibits the highest potency in this example, significantly outperforming Compound 5.

CETSA EC_{50} Correlates with Other Measures of Affinity

Traditional affinity assays primarily measure functional activity, but CETSA provides direct evidence of target engagement within cells. To assess engagement in native conditions, a panel of compounds can be compared across multiple platforms, including CETSA.

Case Study: RIPK1 Inhibitors

Receptor-Interacting Protein Kinase 1 (RIPK1) is implicated in various diseases, including acute and chronic inflammation, multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS).3 Takeda® developed a panel of RIPK1 inhibitors and evaluated 14 compounds using CETSA, assessing target engagement within in vitro cell lines, in vivo dosed mice, and both mouse and human Peripheral Blood Mononuclear Cells (PBMCs).

To compare CETSA derived EC_{50} values with functional assays, the data was correlated with a necroptosis assay. This assay measures cytotoxicity in response to necroptosis inducers in the presence or absence of inhibitors. CETSA EC_{50} values correlated well with IC_{50} values from the necroptosis assay, confirming that CETSA can determine target engagement independent of functional effects.

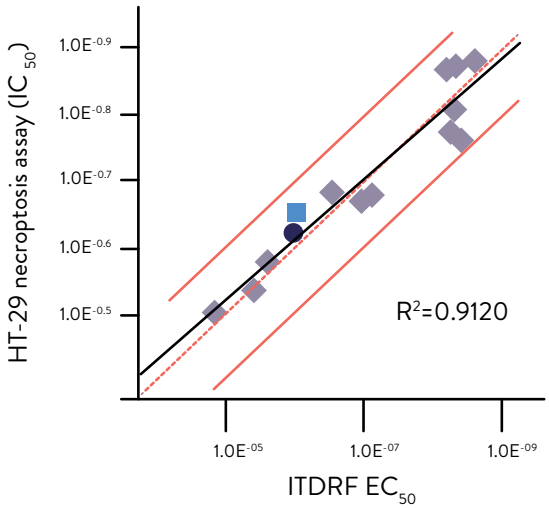


Figure 2. Correlation between functional cellular activity and CETSA: Scatterplot showing IC_{50} values for human HT-29 cells in the necroptosis assay versus CETSA EC_{50} values for endogenous RIPK1 stabilization across 14 RIPK1 inhibitors.

References

Martinez Molina et al. Science 2013
Jafari et al. Nature Protocols 2014
Ishii et al. Nature Scientific Reports 2017
Figures in this application note are modified from original.

PELAGO BIOSCIENCE
The home of CETSA®

CONCLUSION

CETSA derived EC_{50} values provides a relative potency measure for ranking and prioritizing lead compounds in their native physiological environment. By evaluating target engagement in relevant tissues, CETSA accelerates drug discovery and helps mitigate risks and costs before advancing to late-stage trials.

© Pelago Bioscience 2024. All rights reserved. Trademarks and/or registered trademarks mentioned herein, including associated logotypes are the property of Pelago Bioscience AB. This information is not intended to encourage the use of these products in any manner that might infringe upon the intellectual property rights of others.