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The home of CETSA®

HAVE YOU SECURED YOUR CLINICAL TARGET ENGAGEMENT ASSAY?

Demonstrating the use of CETSA® for target engagement studies in whole blood.

Validating a drug's target engagement is essential for linking its mechanism of action to expected clinical effects. In later development stages, the right assay confirms target engagement and enables dose monitoring and accessibility tracking—critical factors for clinical success.

Our CETSA (Cellular Thermal Shift Assay) technology is a powerful tool for assessing target engagement across diverse cellular matrices. Its matrix-agnostic nature allows seamless translation from early preclinical studies to clinical applications, ensuring consistency throughout drug development. Blood is often the preferred matrix for clinical studies and is key in preclinical PK/PD assessments (Figure 1).

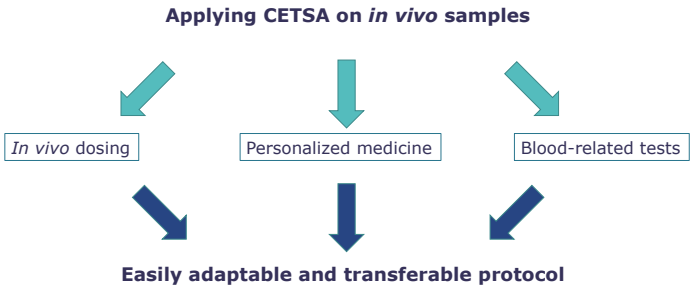

Figure 1. Applying CETSA to complex matrices such as blood and tissue allows for translational studies that complement efficacy and PK/PD studies with target engagement in vivo.

CETSA in Whole Blood Studies

CETSA enables in situ target engagement assessment in both ex vivo and in vivo, providing physiologically relevant insights. The assay accounts for key biological factors, including protein levels, compound uptake, modifications, and sample availability.

In collaboration with AbbVie, we developed a robust CETSA protocol for monitoring target engagement of RIPK1 in whole blood using AlphaLISA™ and MSD® detection methods. This study demonstrates CETSA's ability to assess target engagement in fresh and frozen blood samples (Figure 2). Since clinical samples often require storage and shipping, evaluating assay robustness under these conditions is essential. Additional parameters such as high-abundance proteins (e.g., hemoglobin, albumin, and immunoglobulins) and sample transfer protocols were optimized to ensure assay reliability.

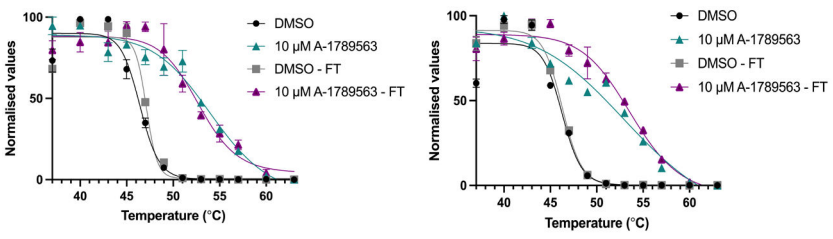

Figure 2. CETSA HT for RIPK 1 +/- inhibitor A-1789563 in fresh and frozen (FT) blood from donor 1 (upper) and donor 2 (lower)

A Target Engagement Assay for Akt in Blood

The serine-threonine kinase Akt is a key regulator of cell survival, proliferation, invasion, and apoptosis, making it an attractive drug target. In November 2023, Capivasertib—a first-in-class Akt inhibitor—received FDA approval for breast cancer treatment.

CETSA-based target engagement assay for Akt was initially developed in K562 cells. After confirming engagement with two reference compounds, the assay was successfully adapted to human whole blood. Analysis revealed donor-specific variations in protein levels and melting behavior (Figure 3). Finally, melt curve shifts were validated using known Akt inhibitors (Bay1125976 and MK-2206), confirming target engagement (Figure 4).

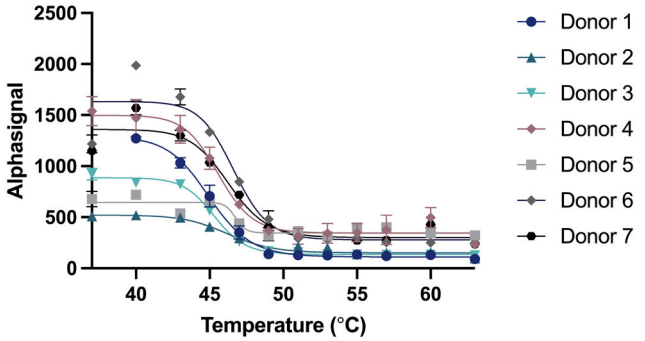

Figure 3. The assay showed donor variations in melting temperatures with Tm varying between 45.0 °C and 46.6 °C.

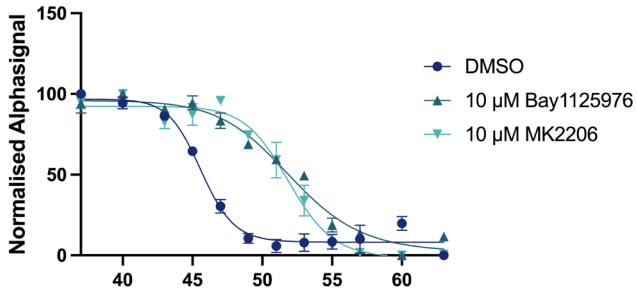

Figure 4. The Akt melt curve was shown to be shifted by the compounds Bay1125976 and MK-2206, indicating target engagement.

References

Song et al. Cancer Research 2019
Patel et al, SLAS Discovery 2024

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CONCLUSION

CETSA is a versatile and scalable solution for target engagement studies in whole blood, supporting a wide range of drug targets. The workflow for assay development in blood involves initial protein detection in a cell line, adaptation to whole blood or PBMCs, and the generation of melt and shift curves to confirm target engagement (Figure 5).

To learn more about using CETSA to expedite your clinical research, **contact Pelago Bioscience today.**

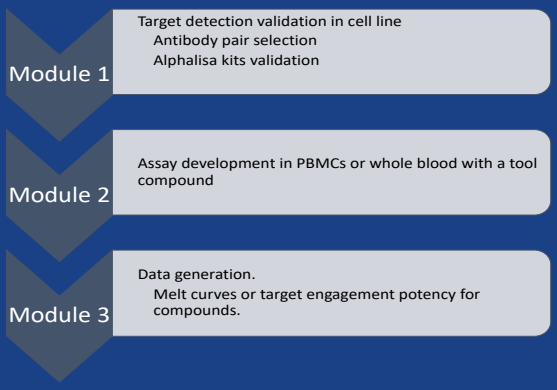

Figure 5. Workflow for CETSA assay development in whole blood.

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