Cellular Thermal Shift Assay (CETSA®) Emerges as a Powerful Tool that Enables Target Engagement Assessment in Preclinical and Clinical settings.

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Understanding target engagement (TE) is crucial for facilitating preclinical and clinical progression by allowing confirmation of on-target effects in complex matrices. While TE can be measured directly or via proximal readouts, challenges persist in establishing assays for reversible small molecule inhibitors, particularly in vivo and in whole blood. This unmet need highlights the importance of identifying a direct TE readout.

While CETSA has been widely used for preclinical characterization of small molecule compounds, its application in unprocessed human whole blood—preferred for clinical studies—has remained unexplored.

This research focused on developing a robust and adaptable **CETSA** protocol applicable across different targets, in whole blood.

Studies of protein-ligand interactions in blood samples pose experimental challenges due to their dynamic nature and inherent variability that assay development must account for. Whole blood samples introduce several complexities due to donor variability, cell composition variations, and experimental setup.

The applicability of the CETSA platform, especially in its high throughput format, makes it amenable to a wide range of targets. Assay development is highly target-dependent and therefore customization is crucial to achieve reliable results.

In summary, this work aimed to streamline CETSA protocols for whole blood, making them more versatile and robust. Addressing blood-specific challenges and adapting the CETSA platform to these has contributed to the understanding of protein-ligand interactions in complex matrices, advancing the CETSA technology toward clinical settings.