

# Repeatability assessment of Tycho NT.6 for better protein quality checks

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## Abstract

Repeatability or the precision at which a measurement is collected is a key consideration when evaluating and interpreting data. Having a strong confidence in the reliability of the results leads to better decision making and more efficient experimental workflows. The Tycho™ NT.6 system provides a quick and precise method to monitor protein quality. In minutes, researchers are able to make better educated decisions on the quality of their sample materials and in turn improve their expression, purification and characterization workflows.

## Introduction and Results

Reproducing results can be the greatest scientific challenge that researchers face. Amazing discoveries can turn into failure if the results can not be reproduced in other scientist's hands.

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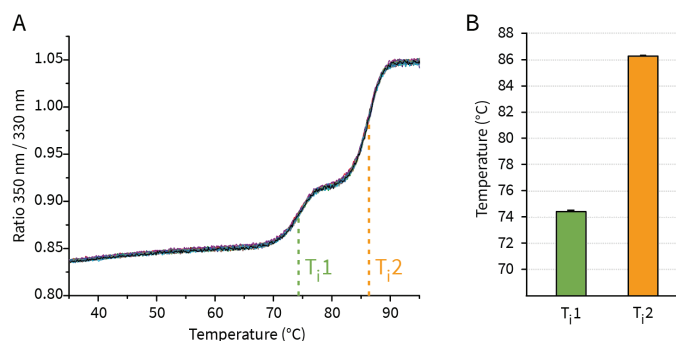
*The first step to generating  
reproducible results is the  
ability to identify and characterize the  
quality of the starting material  
that is being used.*

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Antibodies are an invaluable detection tool used by most researchers working with proteins. Scientists can purchase antibodies from numerous commercial sources as well as generate their own. While validation is provided with commercial reagents including antibodies, often the quality and functionality of the product is poorly controlled and researchers are left to deal with the lack of specificity or modify their experimental plans. On top of this, mishandling and improper storage of antibodies can result in denaturation or reduced specificity which translates into irreproducible results or incorrect conclusions.

Tycho NT.6 provides a rapid and precise way to monitor protein quality within minutes. Requiring only tiny amounts of sample, the thermal stability of any protein sample is swiftly determined. We demonstrate, using trastuzumab as reference antibody sample, the robust repeatability of multiple measurements generated by the system.

Trastuzumab at 2 mg/ml in PBS buffer was repeatedly measured in duplicate over a period of 17 days, storing the sample at 4 °C between measurements. In total, 24 unfolding profiles were recorded (Figure 1A). The repeatability of the two inflection temperatures ( $T_i$ ), or the points on the curve which represent unfolding of the sample due to thermal treatment, was recorded. In addition, the initial fluorescence prior to thermal treatment of the sample was measured in each experiment. (Figure 1B, and Table 1).



**Figure 1: High repeatability of data generated on the Tycho NT.6 system**  
 A) Overlay of 24 unfolding profiles of trastuzumab from duplicate measurements over a time period of 17 days. Two  $T_i$ s of 74.7 °C and 86.2 °C were identified.  
 B) The two inflection temperatures  $T_{i1}$  and  $T_{i2}$  for each measurement were automatically calculated by the Tycho NT.6 software. Error bars represent the standard deviation.

The standard deviation of the results for each identified  $T_i$  were less than 0.01 °C. A strong overlap of the 24 unfolding profiles was observed (Figure 1A). The high precision of the thermal unfolding experiments is illustrated by the negligible deviation of the separate  $T_i$  values throughout the entire measurement period (Figure 1B), as well as by the very small relative standard deviation (RSD) of < 0.1 °C (Table 1). In addition, the day-to-day repeatability and consistency of the fluorescence detection was evaluated by analyzing the initial ratio values. The analysis yielded a standard deviation (SD) < 0.001, demonstrating the exceptional precision of the Tycho NT.6 system optics. The relative standard deviation of the initial fluorescence ratio was 0.11 %.

	Average	SD	RSD (%)
T <sub>i1</sub>	74.4 °C	+/- 0.07 °C	+/- 0.09
T <sub>i2</sub>	86.2 °C	+/- 0.06 °C	+/- 0.07
Initial ratio	0.837	+/- 0.001	+/- 0.11

Table 1: Thermal unfolding profile analysis of trastuzumab over a time period of 17 days. Measurement values represent means from 24 measurements. SD=standard deviation, RSD=relative standard deviation.

## Conclusions

Reproducibility is a common issue faced by all scientists. The Tycho NT.6 system offers high repeatability and precise measurements when examining relative protein stability and quality. In the example shown using the monoclonal antibody

trastuzumab and running 24 replicates, the measured temperature inflection points (T<sub>i1</sub> and T<sub>i2</sub>) had a standard deviation that was less than 0.1°C. This level of precision and repeatability is unmatched by any current technology that examines protein quality. Tycho NT.6 has unrivaled capability to provide accurate and reliable protein stability results, offering researchers an invaluable tool for higher data reproducibility.

## References

1. Freedman, L. P., et. al., The need for improved education and training in research antibody usage and validation practices. Biotechniques, 61: 16-18m 2016.
2. Weller, M.G., Quality issues of research antibodies., Analytical Chemistry Insights, 11:21-27, 2016.