Tech Note
qTOWERiris

# Thermal Shift Assay of β-Lactoglobulin Using CPM on qTOWERiris

## Introduction

The dye 7-diethylamino-3-(4′-maleimidylphenyl)-4-methylcoumarin (CPM) is widely used in protein research to assess stability and solubility, especially for membrane proteins. CPM is non-fluorescent in its unbound form but emits fluorescence upon binding to exposed cysteine residues, which become accessible during temperature-induced protein unfolding. With the availability of the UV-A filter module (Color Module 7), the qTOWERiris 96 UV enables sensitive CPM-based fluorescence measurements within a real-time PCR platform. This expands the application range of the system beyond nucleic acid analysis and into protein characterization.

### Your Benefits

- Broad wavelength spectrum including UV-A range
- Optimized UV filter module for efficient excitation of UVA dyes
- High sensitivity for reliable Thermal Shift Assays
- Ideal for studying membrane proteins

## Application

In this application, CPM-based Thermal Shift Assays (TSA) were used to assess the thermal stability of the model protein β-lactoglobulin under different buffer conditions. All measurements shown were performed on the qTOWERiris 96 UV equipped with color module 7, which provides the optimal excitation and emission wavelengths for CPM ($\lambda_{EX}$ ~ 350 nm, $\lambda_{EM}$ ~ 492 nm). While the method is in principle also compatible with the 384-well version, only data from the 96-well format is presented here. However, the data quality in the 384-well format is comparable and available upon request.

Three protein buffer systems with distinct pH values were tested to evaluate their influence on protein unfolding. As the temperature incrementally increases, structural changes in the protein expose cysteine residues, allowing the CPM dye to bind and emit fluorescence. The resulting fluorescence profiles provide information on the thermal stability and unfolding behavior of the protein under each condition.

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## Results

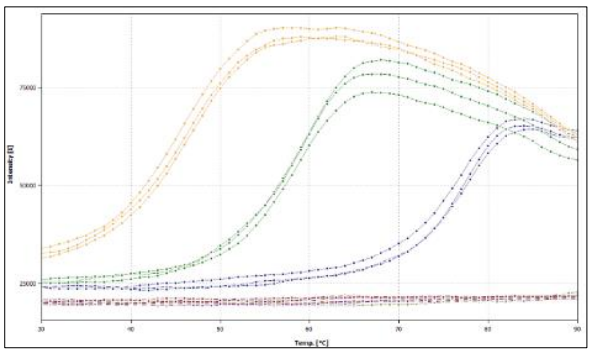

Figure 1: Raw data of melting behavior of protein buffer at three different pH values (4 samples each measured in triplicate)

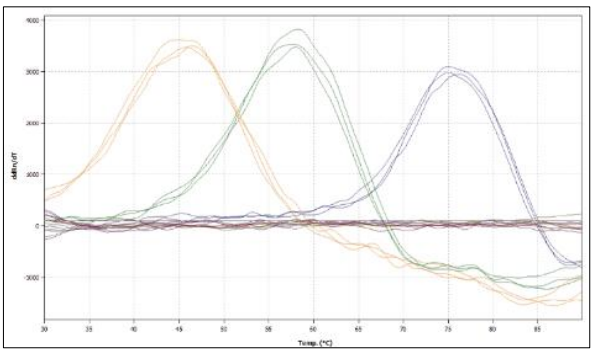

Figure 2: Melting curve calculated by qPCRsoft

Table 1: Melting temperature (Tm) and ΔSD data

| Sample | Tm | ΔSD (Tm) |
|---|---|---|
| pH 5 | 75.43 | 0.75 |
| pH 6 | 58.0 | 0.3 |
| pH 7 | 45.8 | 0.89 |
| NTC | 0 | 0 |

## Conclusion

In this study, melting temperatures (Tm) of β-lactoglobulin were measured at different pH values, revealing a clear influence of buffer conditions on protein stability. While CPM is an established dye for monitoring protein unfolding, this application highlights its successful use on a qPCR platform: the qTOWER iris with UV-A excitation. The system enables sensitive fluorescence detection in both 96-well and 384-well formats, making it suitable for flexible use from standard experiments to high-throughput screening.

Reference: TechNote_qTOWERiris_0022_Thermal Shift Assay CPM_en.docx
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