Introduction

The UNit (*UNchained Labs*) uses up to 3 multi-well cuvettes containing 16 microwells each, monitoring up to 48 samples/conditions in one experiment.

- Determines protein melting temperature (T_m) using tryptophan fluorescence or Sypro Orange and temperature of aggregation (T_{agg}) using static light scattering (SLS) at 266 nm and 472 nm.

Protein Applications of the UNit:

Technical Specifications	
Sample volume	9 µL
Temperature range	15—95 °C
Heating rate	0.01—10 °C/minute
Protein conc. range	0.1—150 mg/mL
SLS laser wavelengths	266 nm, 472 nm
SLS sensitivity	12—22500 kDa · mg/mL
SLS resolution	~ 15 kDa mean
Fluorescence Detector	250—720 nm

- Temperature gradient and isothermal-based experiments
- Optimal buffer screening
- Compound stabilization / destabilization screening and dose response characterization
- Nucleic acid and peptide binding characterization
- Evaluation of protein refolding conditions
- Chemical fingerprinting

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UNit — UNchained Labs

Static Light Scattering and Fluorescence Thermodenaturation

Application Note #1:

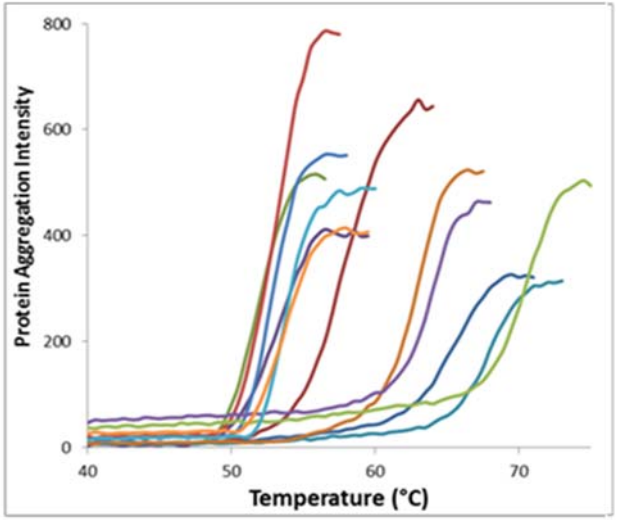
Optimal protein buffer screen

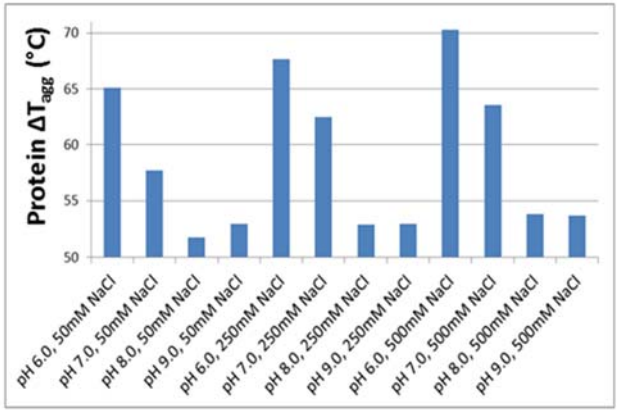
- Greater long/short-term stability
- Reach higher concentration, recover low solubility protein
- Better binding / functional activity
- Promotion of protein crystallization

UNit— Protein Thermodenaturation Assays

Experiment Type 1: Temperature Gradient Denaturation

Protein Sample: 0.5mg/mL, 9 µL
Temperature ramp rate: 1 °C/min, 25 °C to 95 °C
Buffers: 100 mM MES pH 6.0; 100 mM HEPES pH 7.0, 8.0; 100 mM Glycine pH 9.0
Salt: 50, 250, 500 mM NaCl





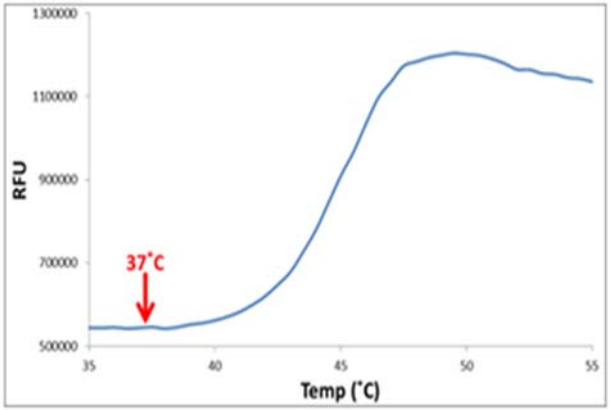
Best: Low pH, High [NaCl]

The protein is stabilized by buffers with lower pH and higher NaCl concentrations.

Experiment Type 2: Isothermal Denaturation

Protein Sample: 0.5 mg/mL, 9 µL
Isothermal denaturation @ 37 °C for 200 min
Buffers: 100 mM MES pH 6.0; 100 mM HEPES pH 7.0, 8.0; 100 mM Glycine pH 9.0
Salt: 50, 250, 500 mM NaCl
Sypro Orange: 5X

Performed initial temperature gradient experiment to determine temperature of denaturation using a midrange buffer of 100 mM HEPES, pH 7.5, 250 mM NaCl.

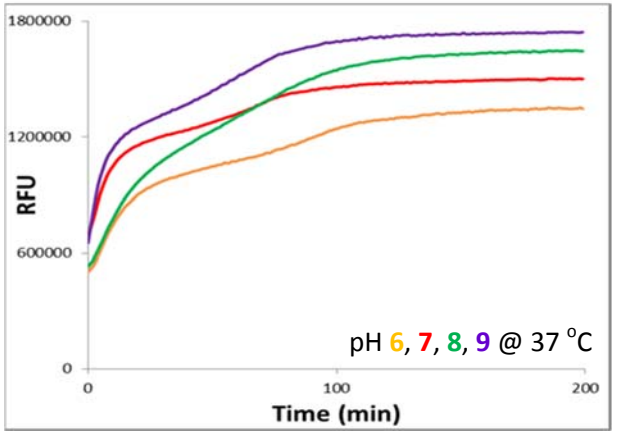


Based on the protein thermodenaturation gradient curve above, an isothermal denaturation temperature of 37°C should be used (2°C below the onset of thermodenaturation at 39°C).

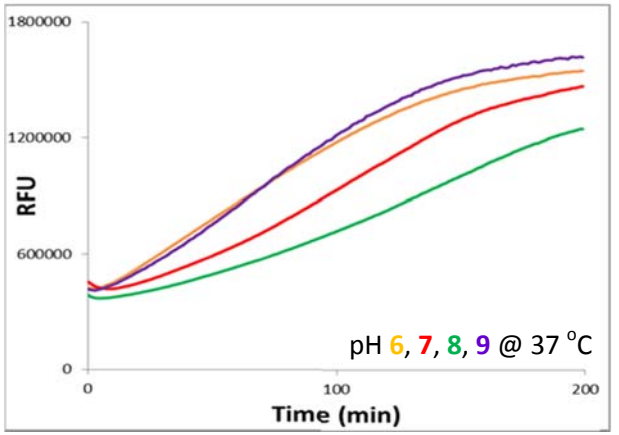
Therefore, the isothermal temperature was held at 37°C for 200 minutes.

The protein is stabilized by buffers with higher NaCl concentrations while no significant changes are observed with varying pH.

Fast Denaturation (50mM NaCl)



Slower Denaturation (250mM NaCl)



Little Denaturation (500mM NaCl)