

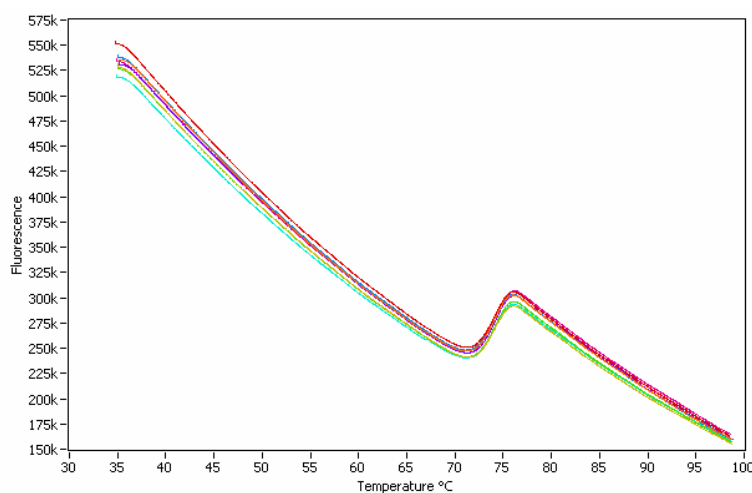
Protein Denaturation Melt Curve Assay

The ability to run precision temperature gradients greatly expands the possible applications for the Genie II platform. User-defined thermal controls allow for highly accurate measurements in thermal shift detection e.g. protein stability/denaturation analysis.

One such method monitors the fluorescence of SYPRO[®]-Orange, which increases when bound to the hydrophobic patches exposed upon protein denaturation. When plotted against temperature, the stability of a protein, or melting temperature (e.g. T_m) can be accurately measured. A high melting temperature indicates high protein stability.

25 μ l reaction containing: 1-10 μ g protein, 1-10x SYPRO[®]-Orange, 1x Buffer (100mM HEPES, 500mM NaCl, pH7-9.0). 35-99 $^{\circ}$ C gradient (0.05 $^{\circ}$ C/sec). Fluorescence is plotted against temperature to produce a melt curve specific to the protein under test.

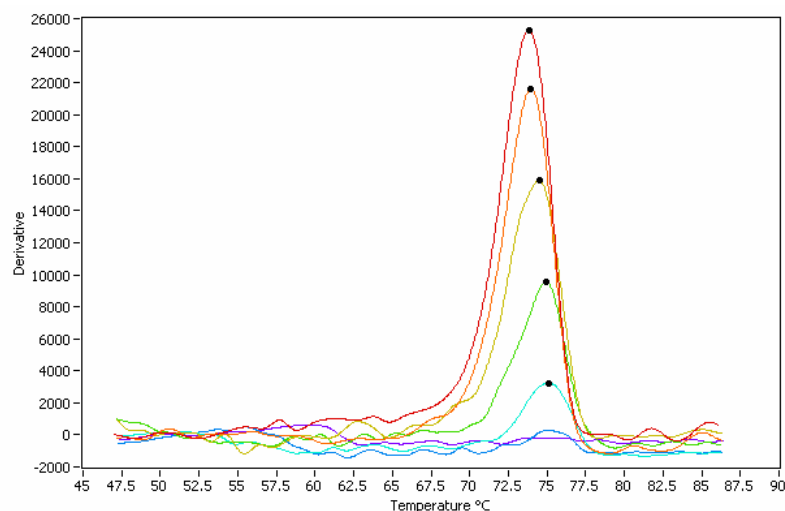
Genie II temperature uniformity:



100mM Hepes , 500mM NaCl pH 7.0	<input type="checkbox"/>
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Derivative plot:

(0.156-10 μ g protein with 0.156x - 10x SYPRO[®]-Orange)



10 μ g - 10x SYPRO	<input checked="" type="checkbox"/>
5 μ g - 5x SYPRO	<input checked="" type="checkbox"/>
2.5 μ g - 2.5x SYPRO	<input checked="" type="checkbox"/>
1.25 μ g - 1.25x SYPRO	<input checked="" type="checkbox"/>
0.625 μ g - 0.625x SYPRO	<input checked="" type="checkbox"/>
0.3125 μ g - 0.3125x SYPRO	<input checked="" type="checkbox"/>
0.156 μ g - 0.156x SYPRO	<input checked="" type="checkbox"/>
NTC	<input type="checkbox"/>