

Protein Thermal Shift DSF Software – Thermo Fisher Scientific

INTRODUCTION

The Lumetics LINK™ software platform scans network locations for new measurement data files, copies data directly to a centralized database, and provides a powerful user interface for rapid multi-measurement multi-technique data aggregation, visualization, analysis, and reporting. LINK employs a client/server-based architecture where the LINK server hardware is provided by the end user and resides on the end user’s network. The LINK client is a portable web-based application that may be placed on any computer with network connectivity to the LINK server. For successful import, the LINK webserver requires read access to the folders where user data resides.

The Protein Thermal Shift Software v1.4 was developed to analyze protein melt fluorescent readings directly from Applied Biosystems™ real-time PCR instrument files. The Protein Thermal Shift Software generates one or multiple melting temperature values (Tm) from these curves by two methods: the Boltzmann-derived Tm and the Derivative Curve-determined Tm, to serve as points of comparison between the curves and represent the relative thermal stability of the protein under different testing conditions.

DETAILS

LINK requires the EDS CSV Experiment Export to import Protein Thermal Shift DSF software exports. Optionally, the Curve Data Export CSV may be imported, however the curve file name must match the EDS.CSV file.

The Protein Thermal Shift CSV data file example is as follows:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	#Positive Hit Settings: dTm Boltzmann: > 2.0, dTm Derivative: > 2.0															
2																
3	#	Well	Protein	Flag Indicator	Tm D	dTm D	Tm2	dTm2	Tm3	dTm3	High Back	Algorithm	Low Signa	Poor Fit	Experiment File Name	
4	1	B02	A-56191 MP1 L3 Temp	1	60.16012		70.9463									LINK.eds
5	2	B03	A-56191 MP1 L3 Temp	1	63.1018		71.78677									LINK.eds
6	3	B04	A-56191 MP1 L3 Temp	1	61.28076		69.82565									LINK.eds
7	4	B05	A-56191 MP1 L3 Temp	1	60.02004		70.10581									LINK.eds

Import Method options can be defined and calculated each measurement during import if it is first created/defined and then requested within an Import Method. More information and instructions can be found in the Users Manual (section 4.27). The following is the default Protein Thermal Shift-specific import method settings:

- Default Sub-Populations: **None**
- Include Edge Particles: **No**
- Image Frame Trimming: **No**
- Time Filter: **None**
- Stuck/Repeating Particle Removal: **OFF**
- Number of Thumbnails to Crop: **250**
- Ignore Thumbnails less than: **5 µm**
- Import Images in order of Largest-to-Smallest: **Yes**

Subpopulations

In addition to the total particle population data set, sub-populations based morphological parameter filters may be generated at the point of measurement import. An unlimited number of sub-populations may be specified for each measurement. For each sub-population, the particle count/concentration vs. morphological parameter will be available, as well as representative particle thumbnail images. The sub-populations do not count towards the measurement limit per project. Additional Sub-Populations will affect import speeds.

When creating as sub-population, select a Morphological Parameter from the pre-defined list and specify the filter criteria. Select the + to add the filter term to the Live Expression View. The Live Expression View can be edited directly. Brackets, mathematical operators/values, logical ANDs or ORs are valid operators. Syntax must be exact, for successful application.



Edge Particles

Include or exclude Edge particles (particles intersecting the edge of the image frame). This selection applies to the total particle population and all defined sub-populations.

Image Frame Trimming

Permits specifying a subsection of the image frame that shall be used to determine particle count and related calculations. A bounding box can be defined to allow the software to either a) include particles within this region (all particles with coordinates up to and including the bounding box are included) or b) exclude particles in this region (all particles outside this bounding box region are included). If a bounding box has been specified, the Volume Analyzed shall be adjusted accordingly for all populations and sub-populations.

Time Filter

Exclude all particles detected at the beginning or end of the analysis. This selection applies to the total particle population and all defined sub-populations.

Stuck/Repeating Particles

Ignore particles images which remain, or move slowly, in successive image frames. This selection applies to the total particle population and all defined sub-populations.

Thumbnail Images

Specify the maximum number of particle thumbnail images to be imported to the LINKdb for each measurement population. Apply a size threshold (the default is 5 microns). Choose whether particles should be first sorted from largest to smallest (based on ECD) or imported in their order of appearance during the analysis. These selections apply to the total particle population and all defined sub-populations.

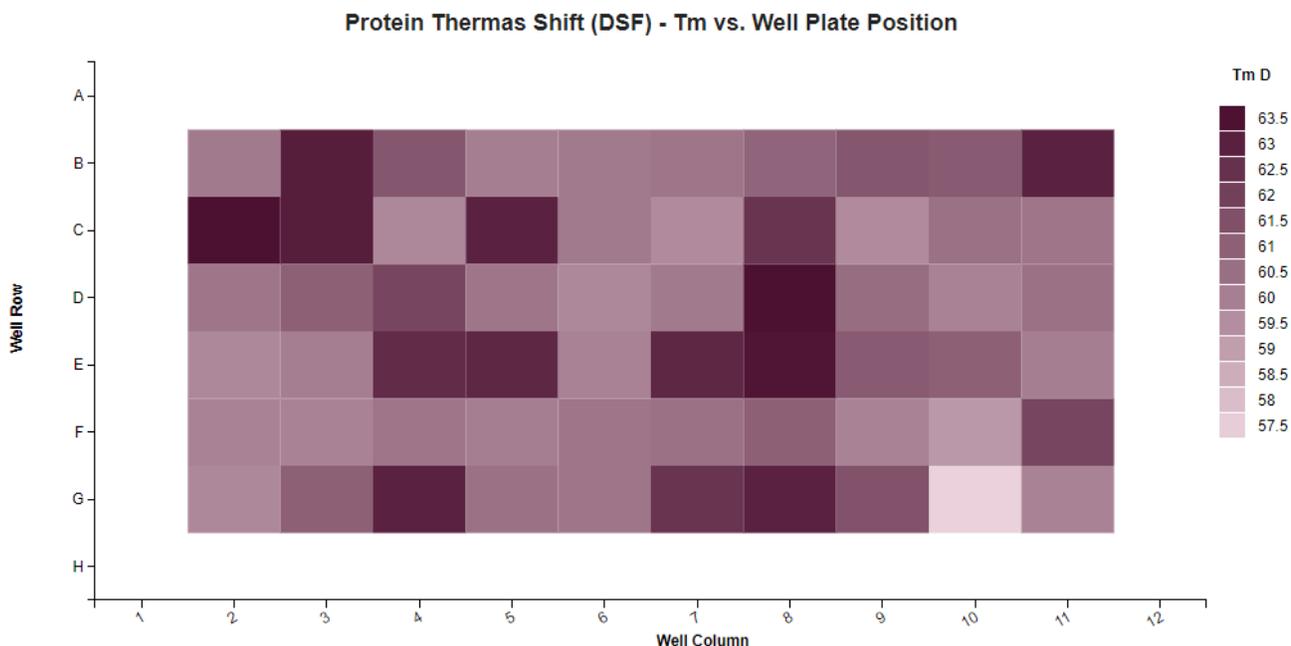
Automated Metadata Extraction

Specify criteria for automated metadata extraction from measurement file fields (e.g., Comments, File Name, Import Path etc...) utilizing specified delimiters. Both metadata name and value may be extracted or only the value only.

EXAMPLES

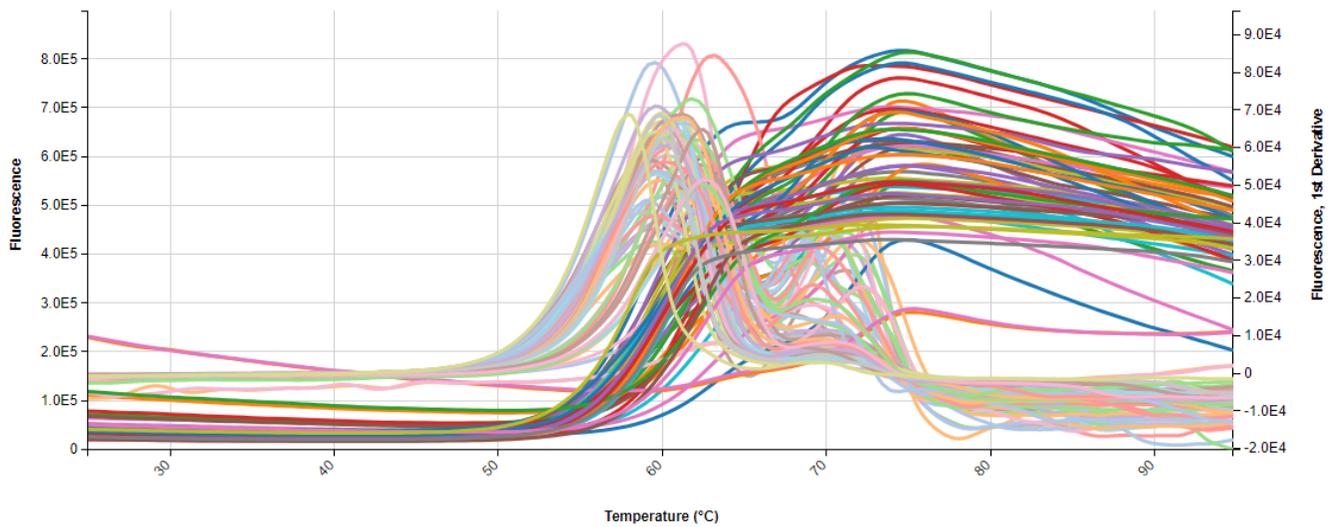
Included below are example dashboards from Protein Thermal Shift DSF Software measurement files:

1. 2D Histogram heat map of well row and well column



2. Line Chart plotting raw data curves for Fluorescence & Fluorescence 1st Derivative vs. Temperature

Protein Thermal Stability: Fluorescence, Fluorescence First Derivative



3. Tabular Summary examples - Measurement Summary Table

Protein	Well	Tm D - COUNT	Tm D - AVG	Tm D - RSD	Tm2 - AVG	Tm2 - RSD	Tm3 - AVG	Tm3 - RSD	dTm D - AVG	dTm D - RSD	Poor Fit	Low Signal
1	Multiple (19 Values)	19	61.39	0.03	70.52	0.02	93.57	0.01				Multiple (2 Values)
2	Multiple (24 Values)	24	60.69	0.02	70.02	0.02						
3	Multiple (17 Values)	17	60.75	0.02	70.00	0.02						

PROTEIN THERMAL SHIFT DSF DASHBOARDS

LINK contains an extensive built-in dashboard library from LINK version 2.4.0.210401 and later. This function contains specific pre-created dashboards for all instruments and application groups.

CONTACT LUMETICS

For direct assistance, please contact Lumetics LINK™ Support:

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