Introduction

The SpectraMax i3x from Molecular Devices is a multi-mode detection system capable of measuring spectral-based absorbance, fluorescence and luminescence.

- Monochromater / filter set hybrid system
- Compatible with 6 to 1536-well assay plates
- Endpoint, kinetic, spectrum, well scan modes
- Temperature controlled environment up to 45°C
- Compressed gas purge environment option

The system is upgraded with additional detection capabilities including:

Fluorescence polarization (anisotropy)
Measure molecular mobility using fluorescein (FITC) dye.

Time-resolved fluorescence energy transfer
Europium / Samarium based to investigate molecular pair distances.

Absorbance	
Wavelength range	230 - 1000 nm
Wavelength bandwidth	4.0 nm
Fluorescence	
Wavelength range	250 - 850 nm
Scan increments	1.0 nm
Excitation bandwidth	9 /15 nm
Emission bandwidth	15 / 25 nm
Luminescence	
Wavelength range	300 - 850 nm
Wavelength selection	1.0 nm increments

General applications of the plate reader

- Functional assays
- Binding assays
- Kinetic and endpoint assays
- Numerous other applications

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SpectraMax i3x Plate Reader

Fluorescence, Luminescence, Fluorescence polarization, TR-FRET & Absorbance Hybrid Microplate Reader

Application Note:

Instrument and Method Validation for Protein Quantification

- Quantification of protein samples using Bradford Reagent
- Determination of the instrument linear dose response range
- General assay validation and optimization

Plate Reader - Protein Quantification Assay Development

Instrument and Method Validation for Protein Quantification

Protein sample: Bovine Serum Albumin (A7906 Sigma-Aldrich), 20mM HEPES pH 8, 150mM NaCl
Concentrations: 0.004 - 1 mg/mL
Reagent: Bio-Rad Protein Assay (#5000006) (Bradford Reagent)
Detection: Abs_{595}
Plate type: 384-well black, clear bottom, low - volume (Corning # 3544)
Sample volume: 10 µL

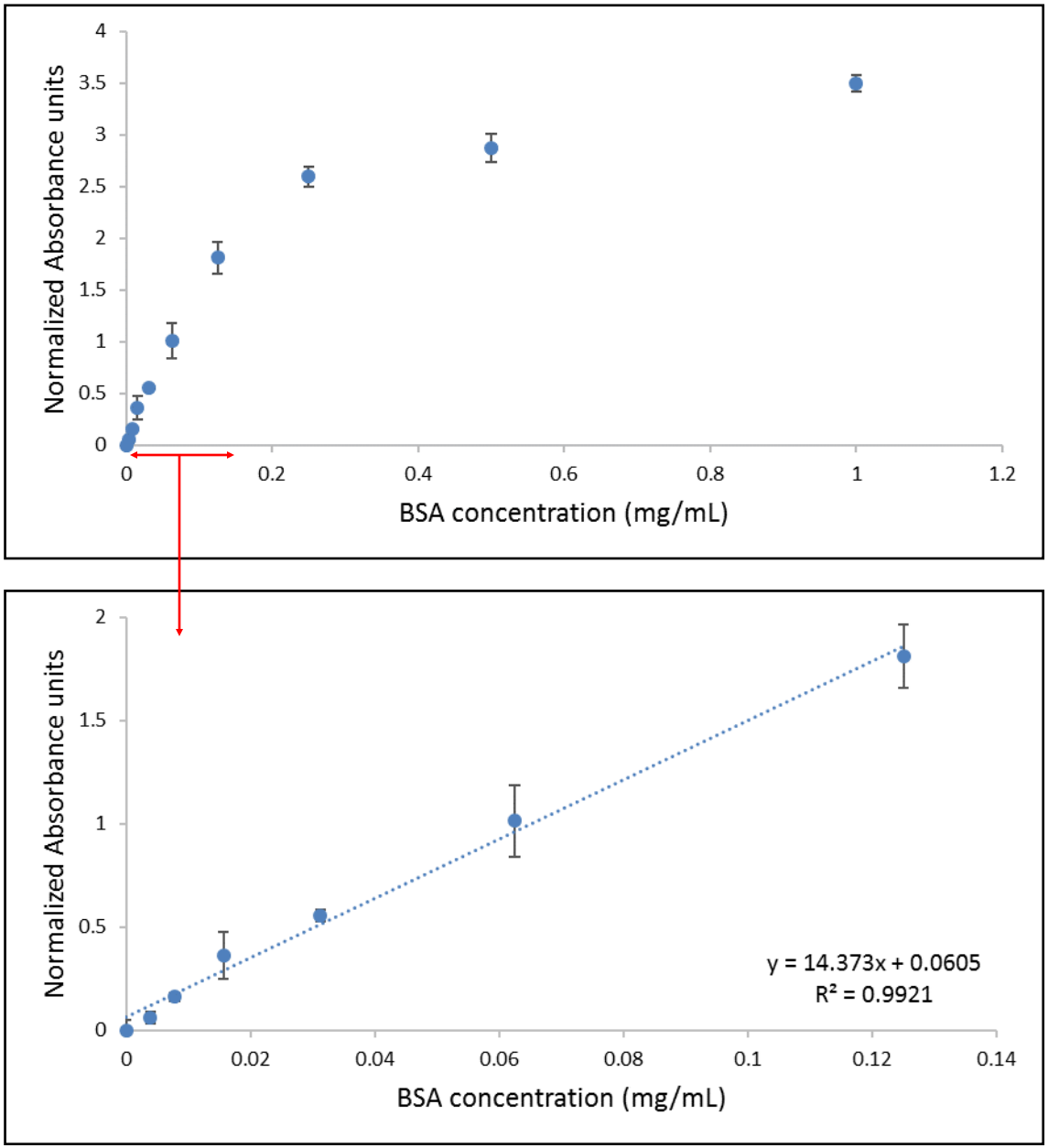
The objective of this exercise is to determine the linear dose response range the assay/instrument.

A stock BSA protein concentration of 1.25 mg/mL was created. To obtain the final concentration, 4 parts of the protein sample was combined with 1 part Bradford reagent (originally 5X concentrated).

Duplicate samples were prepared in the microplate, centrifuged to remove air bubbles and read twice using the same settings to create replicate absorbance values.

Data acquisition was performed using Molecular Devices' SoftMax Pro software. The PathCheck option, which normalizes absorbance readings to a path length of 1 cm for all samples, was used. This reduces errors caused by inconsistent sample volumes leading to different path lengths across the sample wells.

The results show that there is a linear dose response, with an $R^2 = 0.99$, from approximately 0.004 - 0.13 mg/mL protein. The detection response falls outside of the acceptable linear range of the instrument above this data point. This instrument and method validation strategy can be applied to other reagents and assay detection methods for assay validation and optimization.



The linear response range is from 0.004 - 0.13 mg/mL protein using a Bradford reagent and measuring Abs_{595}.