

Comparison of Fluorescence and Multi-Angle Light Scattering for Rapid Quantitation of Bacteriophage

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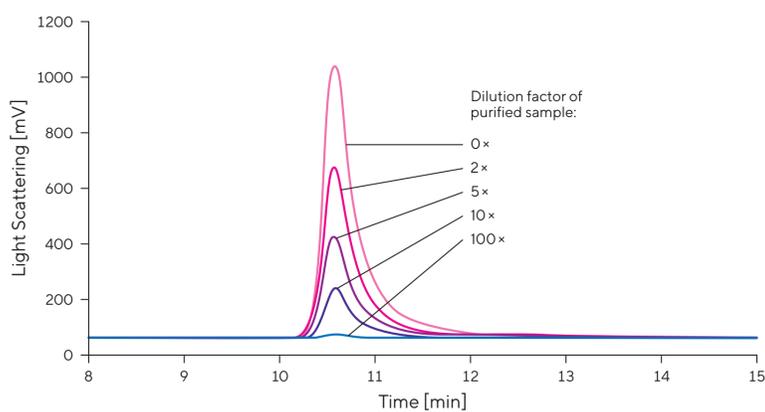
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Introduction

This poster presents a pair of high precision, high accuracy chromatography-based assays that permit determination of phage concentration in less than 1 hour. Sensitivity of UV absorbance is poor because of the low concentration of phages. However, phage sensitivity is strongly amplified by monitoring the chromatogram with either fluorescence or MALS. Fluorescence works by measuring the fluorescence emission from tryptophan residues of the phage proteins. MALS works by passing a laser beam through the sample and reading the scatter produced when it encounters a particle. Larger species generate more scatter.

Results

Multi-Angle Light Scattering



Acknowledgments

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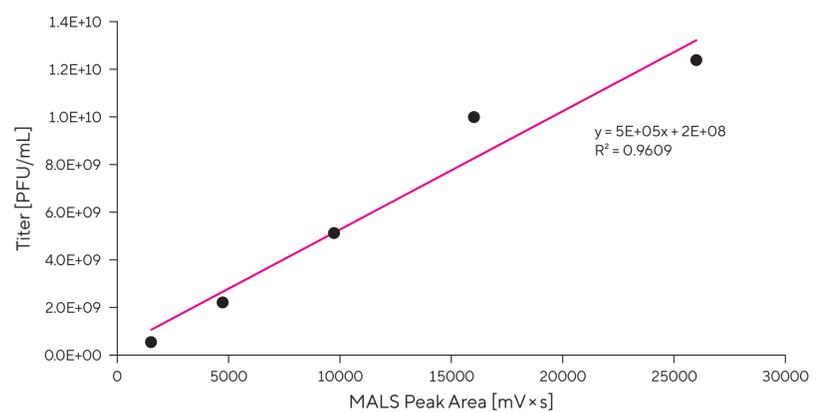


Figure 1: Illustrates a Calibration Curve Generated From a Dilution Series of Purified Phage (T4) And Measured by MALS. The Figure Also Illustrates Values Obtained by Measuring Each of the Dilutions by Plaque Assay (Values in Black).

Fluorescence

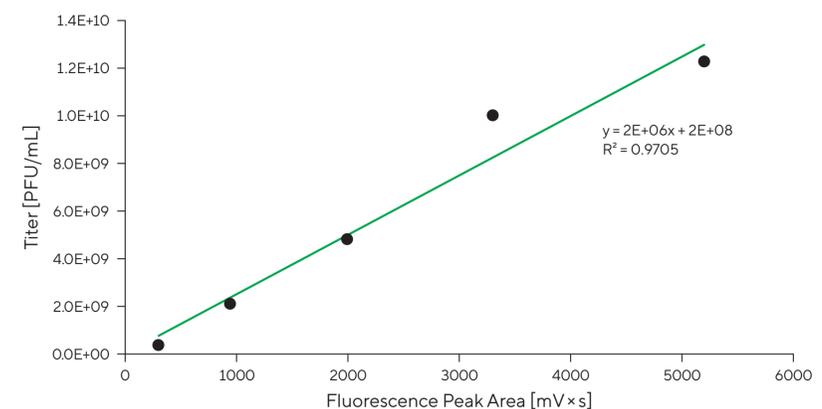
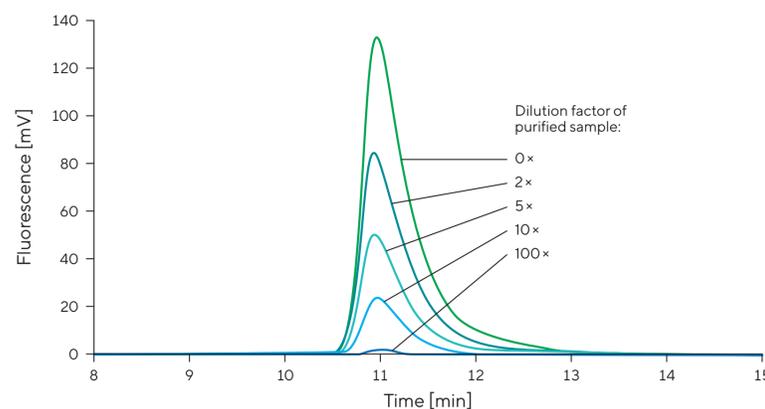
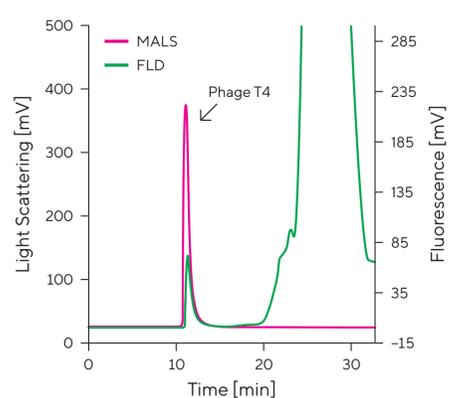


Figure 2: Illustrates a Calibration Curve Generated From a Dilution Series of Purified Phage (T4) And Measured by Fluorescence. The Figure Also Illustrates Values Obtained by Measuring Each of the Dilutions by Plaque Assay (Values in Black).

T4 Harvest



PS09 Harvest

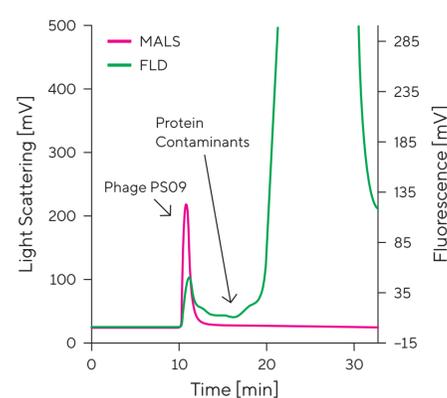


Figure 3: Illustrates the Size Exclusion Chromatography Profile of a Phage-Containing Clarified Cell Culture Harvest of Two Different Phages, Monitored by Fluorescence (FLD) And Multi-Angle Light Scattering (MALS): Harvest of Bacteriophage T4 (Left) And Harvest of Bacteriophage PS09 (Right).

Conditions

- Buffer: 50 mM MES, 150 mM NaCl, 0.05% Poloxamer 188 pH 6.5
- Column: TSKgel® G4000SWXL 8 μm HPLC Column
- LC system: PATfix®, 100 μL sample injection
- Flow rate: 0.5 mL/min
- Detectors: Fluorescence (excitation 280 nm and emission 348 nm); MALS (multi angle light scattering) at 90° angle

The phages (T4, PS09) are too large to enter the pores of the chromatography media so they elute in the void fraction, separate from later-eluting contaminants that are small enough to diffuse into and out of the pores as the sample migrates down the column. Protein impurities are detectable with fluorescence and can interfere and overlap with bacteriophage peak.

Conclusion

- Fast titer determination enables faster evaluation of culture conditions, harvest contents, and phage recovery across purification process steps.
- Both MALS and fluorescence are good for quantification of bacteriophage pure samples.
- MALS may be better for impure samples, because fluorescence can be influenced by contaminating proteins.