JENWAY

SPECTROPHOTOMETER

Cole-Parmer®

Calculations used on Jenway Life Science Spectrophotometers

Introduction

The following tables highlight the calculations used for calculating nucleic acid and protein concentrations when using Jenway Life Science Spectrophotometers such as the Genova Bio, Genova Plus and Genova Nano. The basis of the calculations can also be used to set up an appropriate sum/factor on other instruments which do not have this built in to the software directly.

Nucleic acids

Test Name	Calculation(s)	Default parameters	Displayed result
dsDNA	Conc. = (F x A_{260}) x D_f	260nm Factor = 50 Diluent vol. = 0 Sample vol. = 1	µg/ml
ssDNA/oligo	Conc. = (F x $_{A260}$) x D_f	260nm Factor = 33 Diluent vol. = 0 Sample vol. = 1	µg/ml
RNA	Conc. = (F x A_{260}) x D_f	260nm Factor = 40 Diluent vol. = 0 Sample vol. = 1	µg/ml
DNA allowing for protein contamination (260, 280nm)	Conc. = [F_1 x (A_1-A_{ref})] – [F_2 x (A_2-A_{ref})] x D_f	A_1 = 260nm A_2 = 280nm A_{ref} = 320nm (optional/variable) F1 = 62.9 F2 = 36.0 Diluent vol. = 0 Sample vol. = 1	µg/ml
DNA/RNA purity scan (260/280nm)	Ratio = (A_1 – A_{ref})/(A_2 – A_{ref}))	A_1 = 260nm A_2 = 280nm A_{ref} = 320nm (optional/variable)	Ratio

• Protein

Test Name	Calculation(s)	Default parameters	Displayed result
Direct UV (280nm)	Conc. = $[(A_{280}-A_{ref})/F] \times D_f$	280nm A_{ref} = 320nm (optional/variable) Factor = 1 Diluent vol. = 0 Sample vol. = 1	mg/ml
Warburg-Christian allowing for nucleic acid contamination (280, 260nm)	Conc. = $[F_1 \times (A_1-A_{ref}) - F_2 \times (A_2-A_{ref})] \times D_f$	A_1 = 280nm A_2 = 260nm A_{ref} = 320nm (optional/variable) F_1 = 1.55 F_2 =0.76 Diluent vol. = 0 Sample vol. = 1	mg/ml
Concentration using percent solution extinction coefficient $\varepsilon_{percent}$, the absorbance of a 1% (1g/100ml) solution	Conc. = $[(A_{280}-A_{ref}) \times 10/\varepsilon_{Percent}] \times D_f$ **Note**: default for $\varepsilon_{Percent}$ is a typical average and gives the same result as for Direct UV 280nm. $\varepsilon_{Percent}$ value may be known for a protein however can be calculated as follows: $\varepsilon_{Percent} = \frac{\text{Molar extinction coefficient } (M^{-1}cm^{-1}) \times 10}{\text{MW (g/mol)}}$	280nm A_{ref} = 320nm (optional/variable) $\varepsilon_{Percent}$ = 10 ($g/100ml^{-1}cm^{-1}$) Diluent vol. = 0 Sample vol. = 1	mg/ml

• Protein colorimetric assays

Test Name	Calculation(s)	Default parameters	Displayed result
BCA	Standard curve Unknowns derived from the standard curve depending on fit chosen.	562nm A_{ref} = 750nm (optional/variable) Curve fit = linear Number of standards = 6 Units = µg/ml	Concentration in chosen units
Biuret	Standard curve Unknowns derived from the standard curve depending on fit chosen.	546nm A_{ref} = 750nm (optional/variable) Curve fit = linear Number of standards = 6 Units = µg/ml	Concentration in chosen units
Bradford	Standard curve Unknowns derived from the standard curve depending on fit chosen.	595nm A_{ref} = 750nm (optional/variable) Curve fit = linear Number of standards = 6 Units = µg/ml	Concentration in chosen units
Lowry	Standard curve Unknowns derived from the standard curve depending on fit chosen.	750nm A_{ref} = 405nm (optional/variable) Curve fit = linear Number of standards = 6 Units = µg/ml	Concentration in chosen units
Pierce 660	Standard curve Unknowns derived from the standard curve depending on fit chosen.	660nm A_{ref} = 770nm (optional/variable) Curve fit = linear Number of standards = 6 Units = µg/ml	Concentration in chosen units

OD600

Test Name	Calculation(s)	Default parameters	Displayed result
OD600	$OD600 = A600 \times D_f$	600nm Diluent vol. = 0 Sample vol. = 1	Abs
OD600 with factor	$Cells/ml \times 10^8 = (A600 \times C_f) \times D_f$ **Note**: C_f is a conversion factor used to convert the OD600 reading to cells/ml. Cells should be counted using an appropriate technique. C_f is the gradient of a plot of OD600 vs cells/ml. Default of 5 is based on 1 OD600 = 5×10^8 cells/ml for *E. coli*	600nm Diluent vol. = 0 Sample vol. = 1 $C_f = 5$	n x 10^8 Cells/ml