

A₂₀₅ Custom Method for Protein and Peptide Quantification

Key Words

NanoDrop Spectrophotometer, Custom Method, Peptide Quantification, Protein Quantification

Introduction

Absorbance based protein concentrations are typically determined using the 280 nm wavelength corresponding to tryptophan, tyrosine, and to a lesser extent cysteine residues present in the primary protein sequence. Conversely, protein or peptide sequences absent of these residues can be quantified, in non-absorbing buffer systems, via the Scopes method (Scopes, 1974). This method monitors the absorbance of the peptide bond at 205 nm. The Thermo Scientific™ NanoDrop™ 2000/2000c spectrophotometer utilizes the 205 nm UV wavelength in conjunction with the patented sample retention technology to enable sensitive micro-volume quantitation measurements of peptides and proteins.

Establishing the new Custom Method

1. Launch the NanoDrop 2000/2000c software and select the **Method Editor** button on the main menu.
 2. Enter a name for the method (suggested title: A205 Proteins & Peptides) and select “**Manually entered factor / extinction coefficient.**” Then click on the Next button at the bottom of the window.
 3. Enter the following information below on the **Measurement** tab:
 - Analysis wavelength: 205 nm
 - Result units: mg/mL
 - Decimal digits: 3
 - Choose factor extinction coefficient at 1 cm pathlength: Use extinction coefficient 31 mg/mL

NOTE: The E^{0.1%} (1 mg/mL) value at 205 nm = 31 (Scopes, 1974)
 4. Enter the following information below on the **Correction** tab, check the **Baseline correction** box and enter **340 nm**. Click on the Next button.
 5. Click next past the **Additional Measurements** tab and select the UV range (190–350 nm) on the **Instruments Settings** tab. In addition, check the box to utilize the **Auto pathlength (analysis wavelength)** feature.
 6. Click **Save** in the top left corner of the window and the new custom method will appear in the method list as shown in Figure 1.
- Click on the **Next** button.



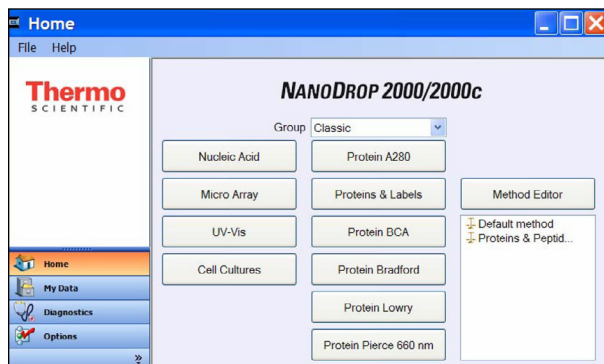


Figure 1: Method List with newly established Proteins & Peptides A₂₀₅ method

The newly established custom method is ready to be used for direct estimation of protein or peptide concentrations using either the pedestal or the cuvette (NanoDrop 2000c spectrophotometer) mode. See Figure 2 for an example of a peptide measured on the NanoDrop 2000 spectrophotometer using the above described custom method.

Reference Literature

Scopes, R.K. 1974. Measurement of protein by spectrophotometry at 205 nm. *Anal. Biochem.* 59:277–282.

Grimsley G. R., and Pace C. N. “Spectroscopic Determination of Protein Concentration” *Current Protocols in Protein Science* (2003) 3.1.1–3.1.9. John Wiley & Sons, Inc.

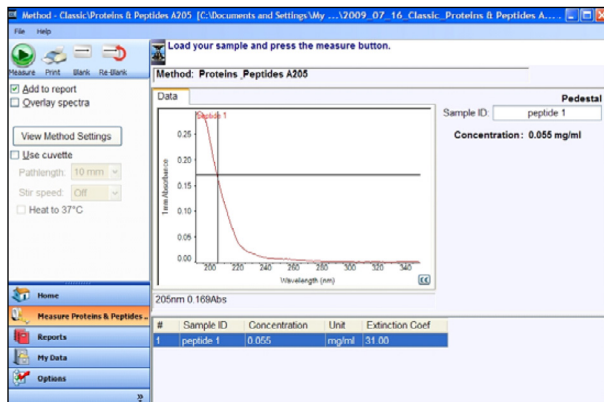


Figure 2: Peptide measurement at 205 nm using a custom method

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