Tryptophan fluorescence (WF) for total protein and peptide determination

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Principle

The fluorescence spectrometry of tryptophan offers a simple, sensitive, and direct method for protein and peptide assays (Wiśniewski & Gaugaz, 2015). The WF assay is fully compatible with SDS and other solutes that are commonly used for the lysis of tissue and cells. The assay can be carried out on a standard fluorescence spectrometer with cuvettes and in a 96-well format using a plate reader. The method is particularly suitable for determination of peptide content in diluted samples.

Material

Reagents/Material	Details
8 M Urea in 10 mM HEPES pH 8.5	Prepare 48g of urea in 100 mL 10mM HEPES, pH 8.5 (NaOH) Note: Don't need to make it fresh
Albumin Standard (Pierce, A56979)	Pierce™ Dilution-Free™ BSA Protein Standards, multichannel pipette compatible. Ready to pipet, BSA: 0.125, 0.25, 0.5, 1, 2, 5, 10 mg/mL
96-well plate (Black, flat-bottomed, polystyrene plates)	For example: Corning CLS3915 Or similar plates  ***Note***: You can reuse the plates after washing them.

Sample Dilution

Sample	Dilution for assay
Total lysate or peptide	• Dilute your sample in the assay buffer to end up in concentration range 0.125-10 mg/mL of the standard

Microplate Procedure

1. Pipette 10 µL of each standard or your sample replicate into a **black** microplate well
    a. Tip: Pipet your standard in the 1st, 2nd and 3rd column, then your samples rather than in rows because the standard has 8 concentrations (including 0 mg/mL)
2. Add 200 µL of the urea assay buffer to each well and mix the plate thoroughly on a plate shaker for 30 seconds.
3. Measure the fluorescence on a compatible instrument
   Note: the signal is stable over hours

Settings

Important: Excitation at 295 nm and emission recorded between 320-400 nm and measurement at 20 °C

Here for example for a Tecan reader:

Mode	Fluorescence Reading	Top	
Excitation Wavelength		295	nm
Emission Wavelength		355	nm
Excitation Bandwidth		5	nm
Emission Bandwidth		20	nm
Gain		100	Manual
Number of Flashes		100	
Flash Frequency		400	Hz
Integration Time		50	µs
Lag Time		0	µs
Settle Time		0	Ms
Z-Position (Manual)		20000	µm

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Reference

- Wiśniewski JR, Gaugaz FZ. Fast and sensitive total protein and peptide assays for proteomic analysis. Analytical chemistry. 2015 Apr 21;87(8):4110-6. PMID: **25837572**. DOI: 10.1021/ac504689z