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Product Information

FluoroProfile® Protein Quantification Kit

Catalog Number **FP0010**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The FluoroProfile® Protein Quantification (FPQ) Kit offers a complete protein quantification assay. The FPQ Kit is significantly more sensitive than existing standard colorimetric measurements (Bradford and bicinchoninic acid assays) and exhibits a larger linear dynamic range than other fluorimetric protein determination kits. The inherent high sensitivity of this technology allows users to simply dilute out potential interfering compounds that may be present in various protein samples. Fluorescence intensity is directly proportional to protein concentration; consequently, large differences in protein concentrations generate commensurately large differences in fluorescence intensity. Moreover, the FPQ Kit generates intuitive results and exhibits enhanced robustness to instrument variability.

This assay exhibits very low protein-to-protein variation, leading to more accurate protein concentration values. The kit can effectively stain glycosylated proteins, phosphoproteins, crosslinked (disulfide containing) proteins, metalloproteins, hydrophobic proteins, and lipoproteins. The FluoroProfile assay is not recommended for quantitating proteins containing heme groups, as inaccurate data will be obtained at protein concentrations above 10 μg/ml.

The fluorescent signal is stable for at least 6 hours without any significant loss in intensity. Results are consistent over a working day and at room temperature. Elevated temperatures do not adversely affect signal.

The FluoroProfile Protein Quantification Kit is well suited to high throughput, micro volume assays and automation, in that there are no time consuming and/or complicated steps involved. Users simply mix 1 volume of sample with 1 volume of working reagent and allow 30 minutes for the reagent to interact with the proteins.

The FluoroProfile Fluorescent Reagent is based on epicocconone,[1] a biodegradable natural product. Accordingly, there are no unique concerns for disposal of the product after use nor is special treatment with activated charcoal, often seen with other fluorophores, required.

Components

FluoroProfile Fluorescent Reagent (Catalog Number F5054)	10 ml
Quantification Buffer (Catalog Number Q0509)	10 ml
BSA Standard (Catalog Number P5619)	2 mg

Equipment Required but Not Provided.

- Pipettes
- Fluorimeter cuvettes; alternatively, black 384 or 96 well plates
- Fluorimeter capable of reading at an excitation wavelength of ~510 nm and an emission wavelength of ~620 nm. Alternatively, a fluorimeter with an integrated monochromater may be used, provided the instrument is capable of operating at an excitation wavelength between 350–450 nm and an emission wavelength of ~615 nm.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

BSA Standard Solution – Dissolve the powder directly in the bottle with water or the same buffer as the sample protein. Prepare serial dilutions of the BSA Standard Solution to obtain a standard curve. A series of 4-fold serial dilutions from 200 μg/ml to ~50 ng/ml is recommended (see Table 1). The detection limit is largely determined by the sensitivity limits of the fluorescence instrumentation employed.

Table 1.
Solution Matrix Used to Obtain a Typical Standard Curve

Tube No.	Water or Sample Buffer	BSA Solution	Final Protein Concentration
1	–	1 Volume 200 μg/ml	200 μg/ml
2	3 Volumes	1 Volume 200 μg/ml	50 μg/ml
3	3 Volumes	1 Volume 50 μg/ml	12.5 μg/ml
4	3 Volumes	1 Volume 12.5 μg/ml	3.125 μg/ml
5	3 Volumes	1 Volume 3.125 μg/ml	781 ng/ml
6	3 Volumes	1 Volume 781 ng/ml	195 ng/ml
7	3 Volumes	1 Volume 195 ng/ml	48.8 ng/ml

Storage/Stability

Store the kit at –20 °C. Bring all reagents to room temperature and ensure solutions are homogenous by mixing gently before use.

After the initial thaw of the kit components, aliquot the reagents into single-use quantities to minimize the number of freeze-thaw cycles.

Procedure

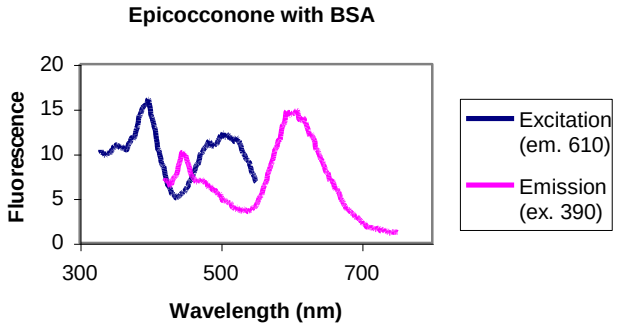
Assay volumes can range from 20 μl in 384 well plates to 200 μl in 96 well plates. The kit works equally well for larger volumes (3 ml in cuvettes). Larger volumes have been shown to increase the upper limit of protein quantification.

The kit is compatible with most industry-standard fluorescent imaging and recording systems that can excite with ultraviolet, blue, or green light, and record red light emission. This includes multiwell plate-based fluorimeters. Laser-based imaging systems are also highly suitable.

Notes: Refer to Interfering Compounds (Specificity section). Interfering compounds in sample buffers used should be at or below concentrations listed in Table 4.

The excitation and emission maxima for the FluoroProfile Fluorescent Reagent (when bound to protein) are 510 nm and 620 nm, respectively. In order to take full advantage of the signal from the fluorophore, it is ideal to choose filters that are spectrally separated and slightly offset from the peak excitation and emission wavelengths.

Figure 1.
Excitation and Fluorescence Emission Spectra



1. Prepare several dilutions of the sample of unknown concentration, using water or sample buffer as a diluent. For example, measure the unknown sample neat and diluted 10, 100, and 1,000-fold in water or sample buffer.
2. Prepare Working Reagent – Mix water, sample buffer, FluoroProfile Fluorescent Reagent (Fluor, Catalog Number F5054) and Quantification Buffer (Catalog Number Q0509) in an 8:1:1 ratio according to Table 2.

Table 2.
Volumes of Working Reagent to Prepare

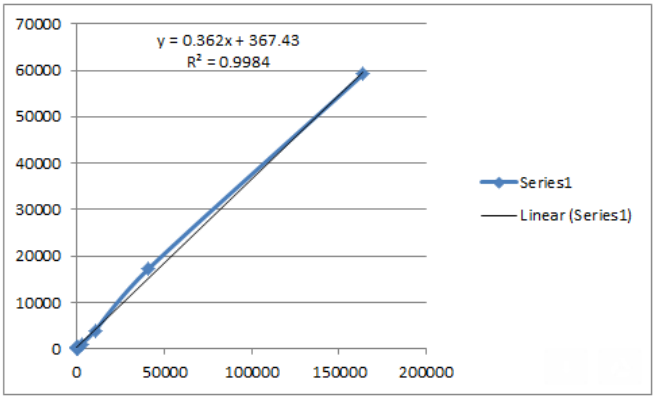
Number of Assays			Volume			
1 ml cuvette assays	100 µl assays in a 96 well plate	20 µl assays in a 384 well plate	Water/ Sample Buffer (µl)	Fluor (µl)	Buffer (µl)	Total volume of Working Reagent (µl)
1	10	50	400	50	50	500
5	50	250	2,000	250	250	2,500
10	100	500	4,000	500	500	5,000
50	500	2,500	20,000	2,500	2,500	25,000
100	1,000	5,000	40,000	5,000	5,000	50,000
200	2,000	10,000	80,000	10,000	10,000	100,000

3. A blank should be prepared by adding equal volumes of Working Reagent and buffer/water.
4. Add an equal volume of Working Reagent to each sample of unknown concentration. The kit is suitable for use with 96 well plates (employing a final volume of 100 µl), hence it is recommended that 50 µl of Working Reagent be mixed with 50 µl of the sample of unknown concentration. Note that larger or smaller volumes may be used if desired.
5. Once the samples of unknown concentration are mixed with the Working Reagent, incubate for 30 minutes prior to measurement of the fluorescent signal. The signal is stable under these conditions for up to six hours. If longer storage periods are required, it is recommended that plates are sealed and stored at 4 °C.
6. Once measurements of the protein standards (at known concentrations) have been obtained, plot fluorescence (y-axis) against protein concentration (x-axis).

Results
The Net RFU is found by subtracting the Relative Fluorescence Units (RFU) obtained for the blank from the recorded RFU values for the protein standards and unknown samples. Create a standard curve (see Figure 2) by plotting the Net RFU versus the protein standard concentrations (ng/ml).

The protein concentration of the unknown sample can then be determined from the Standard Curve.

Figure 2.
Typical Standard Curve



Note: The fluorescence values obtained for the protein standards will likely differ from those presented here. Actual results will vary depending on both the type of fluorescence instrumentation used and sample type.

Calculations
The mass of the protein present in each sample assayed may then be calculated using the following equation:

Mass = Concentration × Volume × Dilution

Mass = sample protein mass (ng)
Concentration = concentration (ng/ml) of sample
Volume = volume (ml) of sample used in the assay
Dilution = dilution factor

Similarly, the total mass of protein present in the undiluted samples of unknown concentration may also be determined. In this instance multiply the determined concentration of the unknown solution by its total volume.

Total Mass = Concentration × Total Volume

Total Mass = total mass of protein (ng) present in sample of unknown concentration
Concentration = concentration (ng/ml) of sample
Total Volume = total volume (ml) of sample of unknown concentration

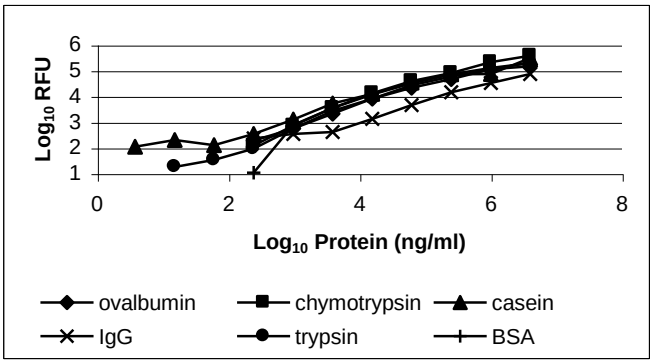
Specificity

Protein-to-Protein Variation

The FluoroProfile Protein Quantification Kit has been tested against a number of different proteins over a wide range of concentrations and minimal variation in response is observed. Only heme-containing proteins were difficult to quantify. Determination of iron-rich proteins is only accurate up to a concentration of 10 μg/ml.

Figure 3.

Fluorescence Signal Variation with Different Proteins



Interfering Compounds

Interfering compounds (see Table 3) should be at or below the following concentrations. Ideally the same buffer should be used for the protein standard and the sample of unknown concentration.

Table 3.

Interfering Compounds

Compound	Concentration
CHAPS	0.05%
SDS	0.1%
Thiourea	500 mM
EDTA	20 mM
NaCl	100 mM
TBP	10 mM
Urea	1 M
TCEP	2 mM
Glycerol	25%
Iodoacetamide	20 mM
2-Mercaptoethanol	20 mM
Sucrose	250 mM
Tris	500 μM
Triton™ X-100	0.002%
TWEEN®	0.005%

Note: This is not a complete list of incompatible compounds. One may assay the protein of interest in ultrapure water alone, then in sample buffer with possible interfering substances. Comparison of the readings will indicate if interference exists. Alternatively, the interfering substance may be removed using dialysis or protein precipitation.

References

1. Bell, P.J.L., and Karuso, P.H., J. Am. Chem. Soc., **125**, 9304–5 (2003).
2. Mackintosh, J.A. *et al*., Proteomics, **3**, 2273–88 (2003).
3. Coghlan, D.R. *et al*., Org. Lett., **7**(12), 2401 -2404 (2005).

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