

Assay kits

FluoProdige

Protein and Peptide Fluorescent Quantification Assay Kit

Protocol

IMPORTANT NOTES – Before you begin

The FluoProdige kit is a ready-to-use assay system for fluorimetric quantification of protein & peptide concentration.

- ✓ The FluoProdige kit presents a complete fluorometric assay for protein and peptide quantification. This kit is more sensitive than classic colorimetric measurements such as Lowry or BCA. It uses a stable analogue to epicocconone molecule that reversibly binds to lysine, arginine and histidine residues in proteins and peptides to yield an intense red-fluorescent product. The fluorescent signal (~518/605) is directly proportional to protein amount among a wide range of protein concentration, rendering this kit highly sensitive.
- ✓ When bound to proteins, excitation and emission signals are 518/605 nm respectively.
- ✓ **We recommend working with Black-well plates (96- or 384-well plates) and alternatively with fluorimeter cuvettes. Avoid the use of white-well plates that would enhance signal and induce background.**

For additional information and protocols (optimization, scaling, co-transfection...) tips, troubleshooting or other applications



www.ozbiosciences.com

Any questions?



tech@ozbiosciences.com

FluoProdige Assay Kit | Specifications

Package content	FPRO200 FluoProdige fluorescent Dye (10 mL) Dilution Buffer (100 mL) BSA (500µg) lyoph Number of assays (96-well plate): 2000 Number of assays (1mL cuvettes): 200
Shipping conditions	The kit is shipped at room temperature.
Storage conditions	Upon receipt, store all reagent tubes at 4°C (up to 6 months). For long term storage, store the dye and BSA at -20°C
Shelf life	1 year from the date of purchase when properly stored and handled
Important notice	For research use only. Not for use in diagnostic procedures.

1. General Considerations

- We recommend adding an equal volume of Working Solution (WS) to sample. Larger volumes of WS have been shown to increase the upper limit of protein quantification.
- Detection limit is determined by the sensitivity limits of the fluorescence reader.

2. Solution preparation

Allow reagent to reach room temperature before beginning, and protect fluorescent dye from light.

Standard Solution.

Dilute the BSA in 1 mL of buffer* or H₂O for a 500 µg/mL standard BSA solution.

Prepare a range of concentrations by two-times serial dilutions. 8 dilutions will result in a range from ~4 µg/mL to 500 µg/mL.

* We recommend using the same buffer than the sample buffer for BSA dilution and standard curve preparation.

Working Solution.

Homogenize the FluoProdige dye by mixing gently before use. Prepare a working solution by diluting the dye and dilution buffer in a 1:9 ratio (refer to table 1 below).

Table 1: Volumes to consider for preparing Working Solution

Number of assays			Volumes		
384-well plate (20µL)	96-well plate (100 µl)	Cuvette (1mL)	Dye (µL)	Buffer (µL)	Total Working solution
5	1	-	5	45	50
50	10	1	50	450	500
250	50	5	250	2250	2500
500	100	10	500	4500	5000

3. General protocol for 96-well plate

1. Standard curve preparation

Prepare 7 tubes containing 50 µL of H₂O or sample buffer

Dilute 50µL of BSA standard into the first tube, and continue with two-times serial dilutions.

Add 10 µL of each standard point to a 96-well plate

Add 40 µL of H₂O.

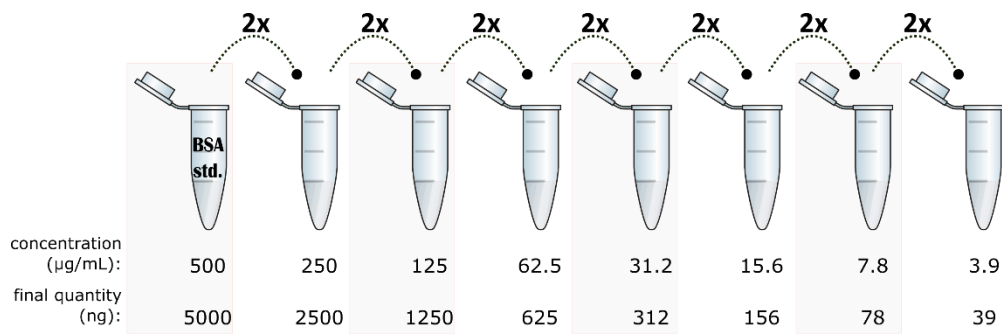


Figure 1: Recommendations for performing serial dilutions using standard BSA. Final quantity (ng) refers to the amount of proteins added into wells.

2. Sample

Add 50 µL of sample to 96-well plate.

We recommend performing at least duplicate; for concentrated samples, dilute 5x or 10x your sample in sample buffer or in H₂O.

Prepare a blank with 50µL of sample buffer or H₂O.

3. Working solution

Add 50 µL of Working Solution to each sample, standard and blank wells

4. Incubate 15 min in the dark at Room Temperature

5. Measurement of fluorescence

Measure fluorescence using a fluorescence microtiter plate reader (exc: ~518nm/ em: ~605nm). Subtract background fluorescence of the blank from all other values.

4. Performance characteristics

Standard curve

Create a standard curve by plotting fluorescence over protein standard amount. For a better visualisation, log₁₀ fluorescence vs log₁₀ protein amount can also be used.

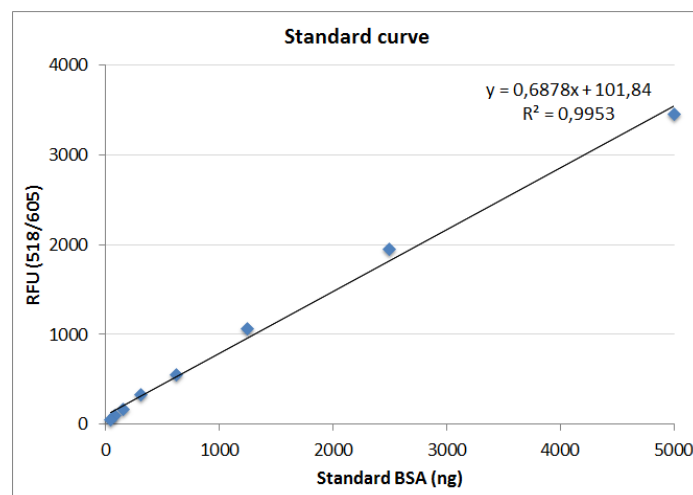


Figure 2: Standard curve realized using serial dilutions of standard BSA

Range of detection

This kit is recommended for detection of low amounts of protein. Linearity in detection is maintained up to 6 µg of protein per sample. However, for high concentrations, we recommend diluting sample to reach linear range.

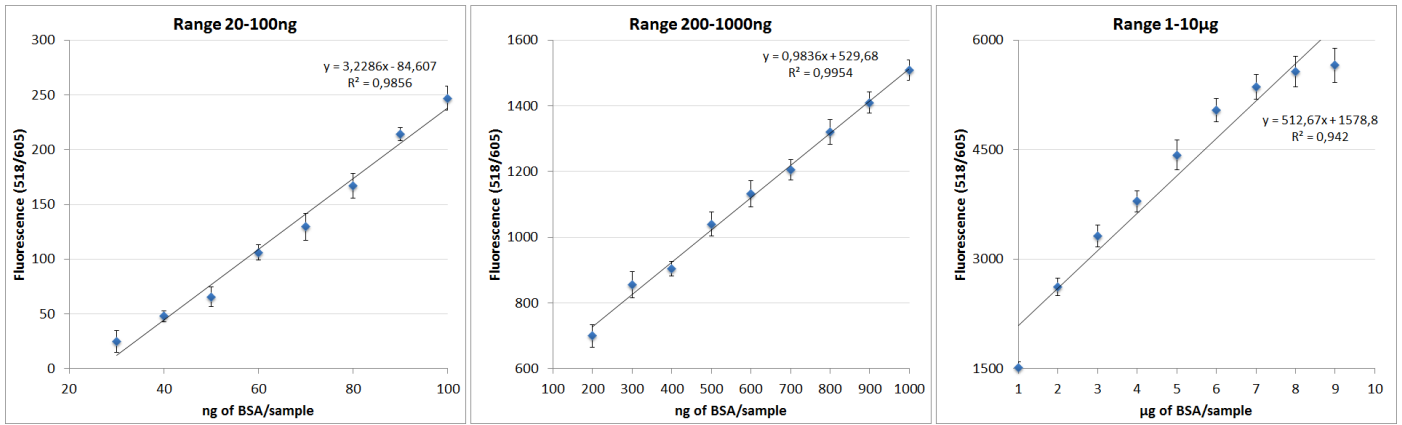


Figure 3: Range of detection of the fluorescent assay kit

Reading Optimization

Time and Working solution volume have been settled for an optimal reading; depending on sample, optimizations can be performed by adjusting time and volume of WS.

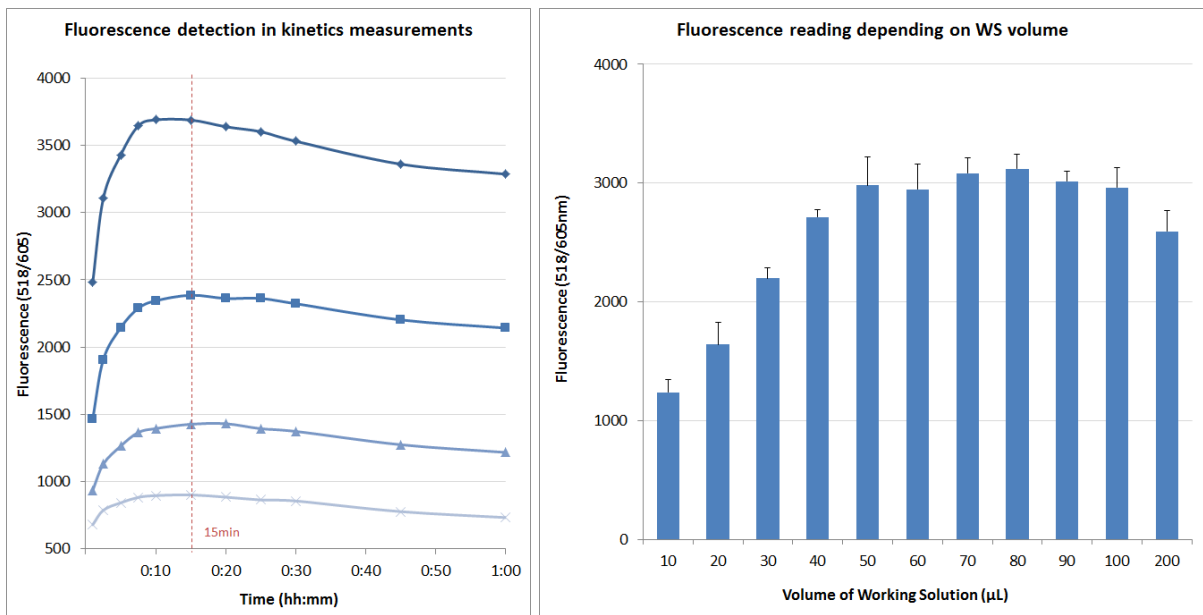


Figure 4: Time (left graph) and WS volume (right graph) optimization for an optimal reading

Interfering compounds

Compounds known to interfere with assay kit should be used at- or below the following concentrations:

This list is not exhaustive. For an optimal reading, we recommend assaying the protein of interest in ultrapure water alone; dialysis or protein precipitation may also be used to remove interfering substance.

Table 1: interfering substances

Compound	Maximum Limit
2-Mercaptoethanol	20 mM
ACN	0.5 %
CaCl	500 μ M
CHAPS	0.05 %
Dithiothreitol	1.5 mM
EDTA	20 mM
Formic Acid	0.01 %
Glycerol	25%
HCl	500 μ M
Iodoacetamide	50 mM
NaCl	100 mM
NH₄CO₃	500 μ M
NP40	0.005 %
SDS	0.1 %
Sucrose	250 mM
TBP	10 mM
TCEP	2 mM
TFA	0.005 %
Thiourea	500 mM
Tris	500 μ M
Triton™ X-100	0.005 %
TWEEN®	0.01 %
Urea	1 M

Additional products for Protein Dosage

- **Bradford Protein Assay kit** for determining the concentration of proteins
- **BCA-PAK Protein Assay Kit** for colorimetric detection and quantification of total protein content

Purchaser Notification

Limited License

The purchase of the FluoProdige Assay kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). This reagent is intended for in-house research only by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the FluoProdige Assay kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all FluoProdige Assay kit reagents and documentation to OZ Biosciences, or by destroying all D-Luciferin components. Purchasers are advised to contact OZ Biosciences with the notification that a FluoProdige Assay kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the FluoProdige Assay kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

FluoProdige Assay kit and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

EUROPE & ASIA OZ Biosciences SAS

163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
France

Ph: +33 (0) 486 948 516
Fax: +33 (0) 463 740 015

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

USA & CANADA OZ Biosciences INC

7975 Dunbrook Road
Suite B
San Diego CA 92126
USA

Ph: + 1-858-246-7840
Fax: + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com



Rev. 04/2021 FB