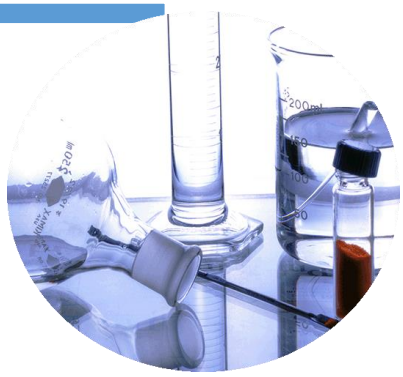
FluoProdige

INSTRUCTION MANUAL

Assays kits



Protein and Peptide Fluorescent Quantification Assay Kit

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

Description	Content	Catalog Number	Number of assays (96-well plate)	Number of assays (1mL cuvettes)
FluoProdige	FluoProdige fluorescent Dye (10 mL) Dilution Buffer (100 mL) BSA (500µg) lyoph	FPRO200	2000	200

For any technical questions, contact us at tech@ozbiosciences.com

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1. Technology

1.1. Description

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.

1.2. Storage and shipping condition

Storage: 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.

Shipping condition: The kit is shipped at RT.

2. Applications and Protocols

2.1. 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.

2.2. 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**

2.3. 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.

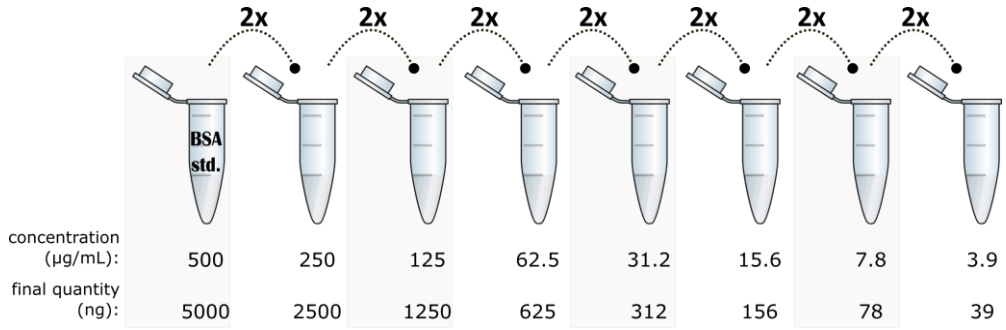

Figure 1: Recommendations for performing serial dilutions using standard BSA. Final quantity (ng) refers to the amount of proteins added into wells.

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.

2.4. 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.

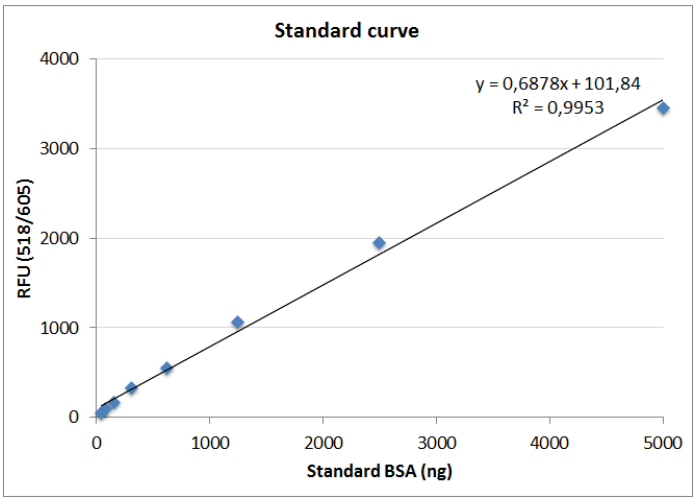

Figure 2: Standard curve realized using serial dilutions of standard BSA

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.

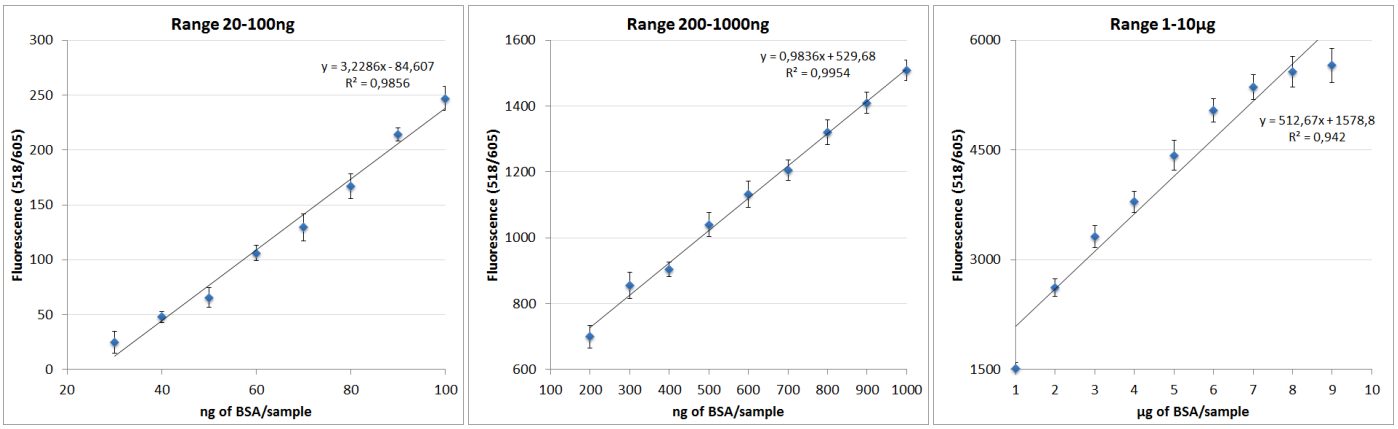

Figure 3: Range of detection of the fluorescent assay kit

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.

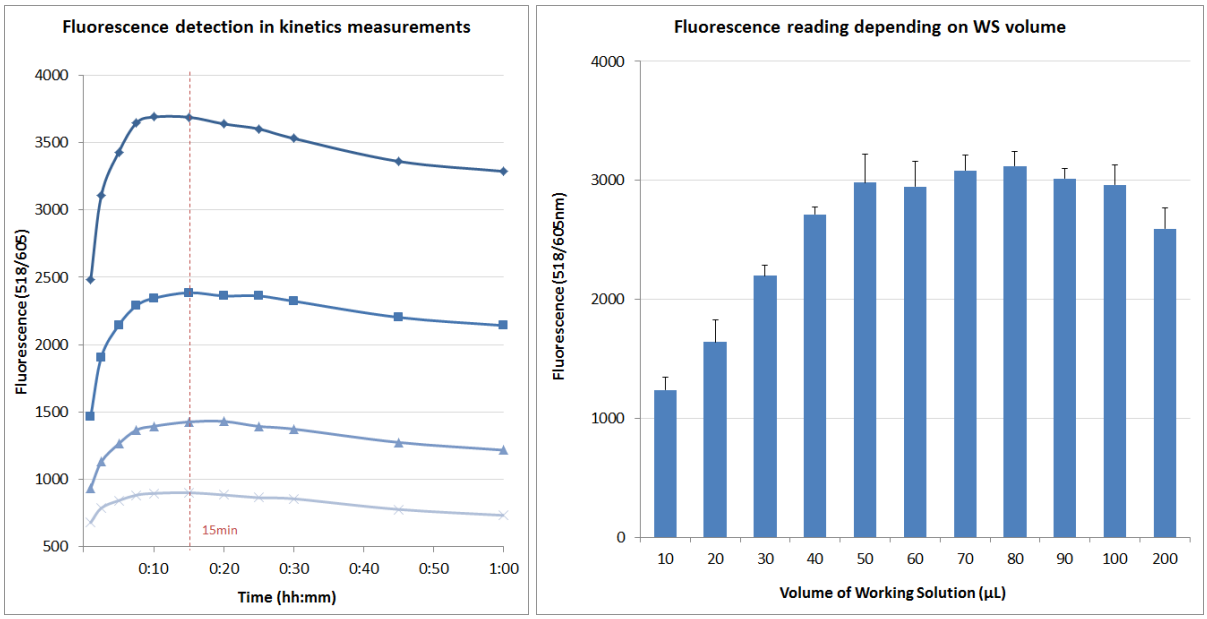

Figure 4: Time (left graph) and WS volume (right graph) optimization for an optimal reading

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.

Compound	Maximum Limit
2-Mercaptoethanol	20 mM
ACN	0.5 %
CaCl	500 M
CHAPS	0.05 %
Dithiothreitol	1.5 mM
EDTA	20 mM
Formic Acid	0.01 %
Glycerol	25%
HCl	500 M
Iodoacetamide	50 mM
NaCl	100 mM
NH4CO3	500 M
NP40	0.005 %
SDS	0.1 %
Sucrose	250 mM
TBP	10 mM
TCEP	2 mM

TFA	0.005 %
Thiourea	500 mM
Tris	500 M
Triton™ X-100	0.005 %
TWEEN®	0.01 %
Urea	1 M

Table 1: interfering substances

3. Related Products

PLASMIDS PVECTOZ
pVectOZ-LacZ
pVectOZ-SEAP
pVectOZ-GFP
pVectOZ-Luciferase
ASSAY KITS
Luciferase Assay kit
OZBlue Cell viability kit
Bradford – Protein Assay Kit
MTT cell proliferation kit
SEAP Assay Kit
X-Gal Staining Kit
Senescence Kit for Stem Cells
β-Galactosidase assay kits (CPRG/ONPG)

NOTES

Purchaser Notification

Limited License

The purchase price paid for FluoProdige Kit by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the section 1, Kit Contents). These reagents are intended **for internal research only** by the buyer. Such use is limited to the use in the product manual. Furthermore, 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.

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