INSTRUCTION MANUAL

AquiPEP

Fluorescent Protein Assay

AQUISTAIN
ECO-FRIENDLY PROTEIN STAINING

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IMPORTANT INFORMATION

The following instructions are for use with **AquiPEP** Protein & Peptide Quantification Kit.

STORAGE INFORMATION

Store **AquiPEP Dye** in a fridge at + 4°C (up to 6 months) in the original brown bottle provided and protect from light. For long term storage, store the dye in a freezer at – 15°C to – 30°C.

WARNINGS AND PRECAUTIONS

- **AquiPEP** Protein & Peptide Quantification Kit is for research use only
- Refer to MSDS for additional safety information
- The product is guaranteed to be free of manufacturer defect, and to function as described when the enclosed protocol is followed by properly trained personnel.

SAFE HANDLING AND DISPOSAL

AquiPEP Protein & Peptide Quantification Kit is an eco-friendly safe solution and requires no special disposal.

Note: All chemicals should be considered potentially hazardous. This product should only be handled by those persons trained in laboratory techniques, and used in accordance with the principles of good laboratory practice. Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. **AquiPEP** is a dilute solution of a synthetic organic dye in DMSO. The diluted working solution is minimally hazardous and non-flammable; however the complete properties of the dye component have not been fully investigated.

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1. KIT CONTENTS

AquiPEP Fluorescent Protein Assay, 10 mL, is sufficient for 2 000 assays.

2. SHIPPING AND STORAGE CONDITIONS

Shipping at ambient temperature (below 25°C) is acceptable if the total dispatch time is no longer than 5 days. Upon receipt, store **AquiPEP Dye** in a fridge at + 4°C (up to 6 months) in the original brown bottle provided and protect from light. For long term storage, store the dye in a freezer at – 15°C to – 30°C.

3. ADDITIONAL MATERIALS REQUIRED

- High-purity water (distilled, Milli Q or equivalent).
- Bicarbonate buffer (0.1 M Sodium Bicarbonate-Sodium Carbonate Buffer, pH = 9).
- Microcentrifuges tubes 1.5 mL.
- Pipettes.
- Black 384 or 96 well plates; alternatively, fluorimeter cuvettes.
- Fluorescence plate reader capable of reading at an excitation wavelength of ~518 nm and an emission wavelength of ~605 nm.

4. ABOUT AQUIPEP

AquiPEP Protein & Peptide Quantification Kit offers a complete protein quantification assay. **AquiPEP** is significantly more sensitive than existing standard colorimetric measurements (ninhydrin, Lowry, BCA). **AquiPEP** is an eco-friendly fluorescent dye that covalently and reversibly binds to lysine, arginine and histidine residues in proteins and peptides to yield an intensely red-fluorescent product. This unique mechanism allows highly sensitive quantification of proteins and peptides over a wide linear dynamic range. Fluorescence intensity is directly proportional to protein concentration; consequently, large differences in protein concentrations generate commensurately large differences in fluorescence intensity. Moreover, **AquiPEP** Protein & Peptide Quantification 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. **AquiPEP** can effectively stain glycosylated proteins, phosphoproteins, crosslinked (disulfide containing) proteins, metalloproteins, hydrophobic proteins, and lipoproteins.

5. Excitation and Emission Spectra

The excitation and emission maxima for **AquiPEP** (when bound to protein) are ~518 nm and ~610 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. The excitation and emission spectra of **AquiPEP** can be seen in Figure 1.

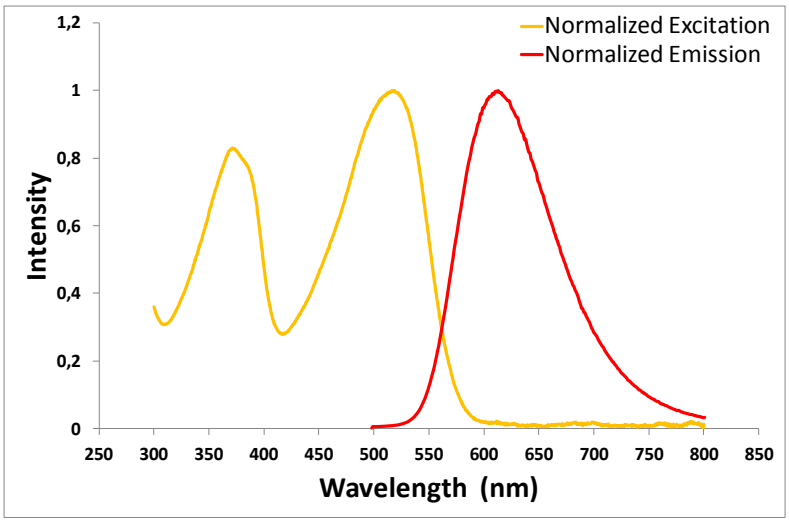

Figure 1. Excitation and Emission Spectra of **AquiPEP** Dye with BSA.

6. Procedures

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 cuvette). Larger volumes have been shown to increase the upper limit of protein quantification. We recommend using 96 well plates with a final volume of 100 µL.

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 multiwall plate-based fluorimeters. Laser-based imaging systems are also highly suitable.

Note: The detection limits is largely determined by the sensitivity limits of the fluorescence instrumentation employed.

7. PREPARATION INSTRUCTIONS

Before assays, prepare Bicarbonate Buffer, Peptide Standard Solution and Working Reagent as described below. Except for Bicarbonate Buffer, solutions should be freshly prepared.

Bicarbonate Buffer

Prepare a 0.1 M solution of Sodium Bicarbonate-Sodium Carbonate Buffer.

The pH of the Bicarbonate Buffer should be approximately pH = 9.

Peptide Standard Solution (to Obtain Standard Curve)

Prepare serial dilutions of a protein or peptide in water or sample buffer (e.g. a 4-fold serial dilution ranging from 0.655 mg/mL to 40 ng/mL is recommended. See **Table 1** for preparing a 4-fold serial dilution).

The peptide standard solution should be prepared using BSA or the same species and buffer as the sample peptide to be quantified.

Working Reagent

If the **AquiPEP Dye** has been stored at – 20°C, allow warming to room temperature and ensuring solution is homogenous by mixing gently before use. Prepare a working solution of AquiPEP fluorescent reagent by mixing dye and bicarbonate buffer in a ratio of 1:9 (See **Table 2** for appropriate dilutions).

Table 1. Preparation of a 4-fold serial dilution to Obtain Peptide standard Curve

Tube N°	Water or Sample Buffer	Protein / Peptide or BSA Solution	Final Protein Concentration of Standard Solution
1	-	1 Volume 655 360 ng/mL	655 360 ng/mL
2	3 volumes	1 Volume 655 360 ng/mL	163 840 ng/mL
3	3 volumes	1 Volume 163 840 ng/mL	40 960 ng/mL
4	3 volumes	1 Volume 40 960 ng/mL	10 240 ng/mL
5	3 volumes	1 Volume 10 240 ng/mL	2 560 ng/mL
6	3 volumes	1 Volume 2 560 ng/mL	640 ng/mL
7	3 volumes	1 Volume 640 ng/mL	160 ng/mL
8	3 volumes	1 Volume 160 ng/mL	40 ng/mL

Table 2. Volumes of AquiPEP Working reagent to prepare

Number of Assays			Volume to Prepare		
1 mL cuvette assays	100 µL assays in a 96 well plate	20 µL assays in a 384 well plate	Dye (µL)	Bicarbonate Buffer (µL)	Total volume of Working Reagent (µL)
1	**10**	50	50	450	**500**
5	**50**	250	250	2 250	**2 500**
10	**100**	500	500	4 500	**5 000**
50	**500**	2 500	2 500	22 500	**25 000**
100	**1 000**	5 000	5 000	45 000	**50 000**
200	**2 000**	10 000	10 000	90 000	**100 000**

8. ASSAY PROTOCOL

Step	Notes
1. Blank	
• A blank should be prepared by adding equal volumes of Working Reagent and Water/Buffer.	• For a microtiter plate (100 µL) assay, add 50 µL of sample/buffer/standard and 50 µL of **AquiPEP** working reagent. • Note that larger or smaller volumes may be used if desired.
2. Protein Standard	
• Add an equal volume of Working Reagent to each Peptide Standard Solution.	• 8 solutions at know concentrations to obtained standard curve
3. Unknown sample	
• Add an equal volume of Working Reagent to a sample of unknown concentration	
4. Incubate	
• Incubate in the dark for 30 minutes at room temperature.	
5. Measurement of Fluorescence	
• Measure fluorescence using a fluorescence microtiter plate reader (λexc ≈ 518 nm and λem ≈ 605 nm). • Subtract background fluorescence of the blank from all other values.	• The signal is stable under these conditions for up to 6 hours. • If longer storage periods are required, it is recommended that plates are sealed and stored at 4°C.
6. Standard curve	
• Create a standard curve by plotting fluorescence over protein standard concentrations (log10 fluorescence vs log10 protein concentration).	• Note that a linear fit is normally used, but a larger dynamic range can be achieved with an exponential fit.
7. Results	
• Use the standard curve to determine the protein concentration of the unknown sample.	

9. TIPS AND TROUBLESHOOTING

- **AquiPEP** Protein & Peptide Quantification Kit reacts with primary amines and these should be avoided in your samples and buffers.
- Use high-grade chemicals and freshly prepare any reagents that are unstable.
- **AquiPEP** Protein & Peptide Quantification Kit is suitable for quantification of most proteins and peptides but individual standard curves are required for each peptide.
- Ideally the same buffer should be used for the protein standard and the sample of unknown concentration.
- Prepare fresh working reagent in each assay.
- Use microtiter plates that are suited for fluorescence measurements.

10. INTERFERING COMPOUNDS

Interfering compounds (see **Table 3**) should be at or below the following concentrations.

Table 3. Interfering Compounds.

Compound	Maximum Limit
2-Mercaptoethanol	20 mM
ACN	0.5 %
CaCl	500 µM
CHAPS	0.05 %
Dithiothreitol	1.5 mM
EDTA	20 mM
Formic Acid	0.01 %
Glycerol	25 %
HCl	500 µM
Iodoacetamide	50 mM
NaCl	100 mM
NH_4CO_3	500 µM
NP40	0.005 %
SDS	0.1 %
Sucrose	250 mM
TBP	10 mM
TCEP	2 mM
TFA	0.005 %
Thiourea	500 mM
Tris	500 µM
Triton™ X-100	0.005 %
TWEEN®	0.01 %
Urea	1 M

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.