



NT-CH4191



Peptide & Protein Quantification Kit, with RED EpicoccoStab

for the accurate and sensitive determination of peptides, as well proteins

Product Description

Name:	Protein&Peptide RED EpicoccoStab Fluorescent Assay Contains EpicoccoStab 10X reagent (patented synthetic analogue of epicocconone), 10mL (#1K952d) BSA standard solution 2mg/mL, 10x1mL (#UP36859A).
Catalog Number:	CH4191 , 1 kit up to 2000 assays
Detection:	Excitation wavelengths: 405, 500nm. Emission wavelengths: 560-610/630nm
Storage:	Store at +4°C (up to 6 months) and protect from light. ^(K) For long term storage, store at -20°C. Stable at room temperature for short term. ^(M/L)

Introduction

Protein & Peptide quantification is prerequisite in many areas of proteomics and peptidomics. Colorimetric assays (ninhydrin, Lowry, BCA) often lack the sensitivity to accurately quantify peptides. Amino acid analysis is expensive, often inconvenient, and sensitive to many interfering compounds.

EpicoccoStab Protein & Peptide Quantification is a fluorescence-based quantification kit. It is based on a small, patented modified fluorescent compound¹⁰ derived from epicocconone core that reversibly binds to lysine, arginine, and histidine residues in peptides, and responds to hydrophobic environments, yielding an intense red-fluorescence². This Epicocconone core dye provides superior stability of signal, allowing highly sensitive quantification of proteins and peptides over a wide linear dynamic range. The staining mechanism does not affect proteins, enabling the same sample to be used for quantification and downstream analysis (e.g. MS, HPLC and Edman chemistry).

Facts & Features

FluoProbes® EpicoccoStab Assay Kit:

- can be used with excitation at 405-500nm, and emission at 560-610nm
- is **sensitive to 100 ng/mL** (peptide) and 40ng/mL (proteins).
- has a **large linear wide dynamic range** between 100ng/mL and 160µg/mL (over 3-orders of magnitude)
- accommodates a wide range of convenient assay volumes: 100µL - 3mL
- has low peptide-peptide or protein-protein variability, improving accuracy for complex samples
- signal is not affected by light or temperature and remains stable for up to 6 hours
- suits a wide range of fluorescence measuring instruments
- is **easy to use**: simply mix 1 part working solution with 1 part of sample.
- is quick - data can be read within 60 mins
- is **safe & environmentally friendly**: biodegradable dye, free of strong solvents and acids
- requires no heating steps or time-consuming heating and reduction steps.
- is **compatible with downstream analysis** such as 1-D and 2-DGE, MS and HPLC...
- is robust to many interfering compounds such as DNA, solubilisation reagents and reducers
i.e. no or very low interference was found after addition of monosaccharides or polysaccharides (glucose, dextran or alginate) up to 2mg/mL ([ref.](#))
- is suitable for measuring peptides from proteolytic digestions and most pure peptides.
- does not precipitate or denature protein and peptides that can be used in subsequent assays
- is amenable to N-term sequencing and to functional assays.
- is more robust than other peptide assays and more cost effective than Amino Acid Analysis.
- is ideal for high throughput analysis

Directions for use

Safe Handling and Disposal

EpicoccoStab Protein & Peptide Quantification Kit is an environmentally safe solution and requires no special disposal procedures.

Note: All chemicals should be considered potentially hazardous. This product should only be handled by persons trained in laboratory techniques, and used in accordance with the principles of good laboratory practice. Wear suitable protective clothing including lab coat, safety glasses and gloves.

EpicoccoStab reagent is a dilute DMSO solution of a natural organic dye. The dilution buffer contains bicarbonate buffer with SDS and acetonitrile that may cause mild irritation to eyes. The diluted working solution is non-flammable.

The complete properties of the dye component have not been fully investigated.

Tips and Troubleshooting

- Ensure that you follow the protocol outlined below.
- Avoided primary amines in your samples and buffers, because Epicoccostab may react with.
- Use high-grade chemicals and freshly prepare any reagents that are unstable.
- EpicoccoStab reagent is suitable for quantification of most proteins and peptides but individual standard curves are required for each peptide.
- Prepare fresh Epicoccostab reagent in each assay (see protocol below).
- Use microtiter plates that are suited for fluorescence measurements.

Kit component

EpicoccoStab Protein & Peptide reagent consists of a concentrate of the fluorescent dye.
BSA standard is a solution of Bovine Serum Albumine calibrated at 2mg/mL.

Reagents and Equipment not provided

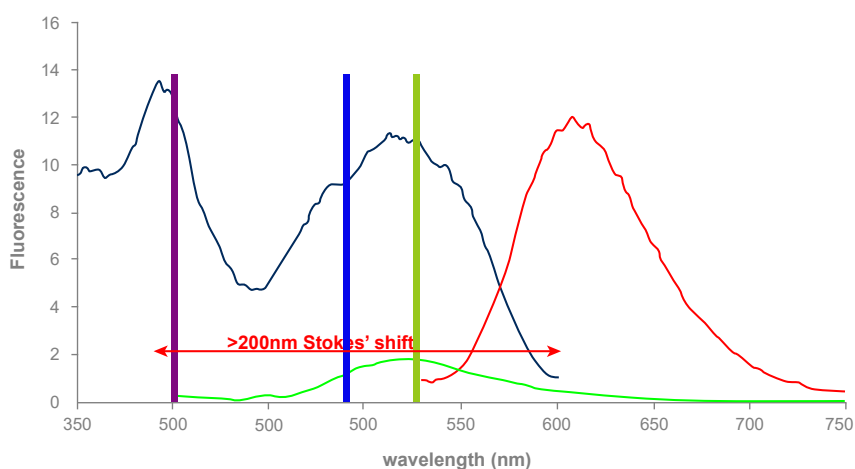
- Bicarbonate buffer (0.1M Sodium Bicarbonate-Sodium Carbonate Buffer, pH 9.0, #R16490)
- Reverse Osmosis water
- Microcentrifuge tubes 1.5mL
- 96-well plate (black, flat-bottom)
- Fluorescence plate reader, with an excitation filter (540±10nm) and an emission filter (630±10nm)

Detection

Excitation: Optimum wavelengths at 405, 500nm.
Suitable light sources include green (e.g. 543, 532nm) blue (e.g. 488nm); violet (e.g. 405nm) or UVA (400nm).

Emission: The maximum emission wavelength is at 610nm, irrespective of the excitation source.
Suitable filters include the 610nm band pass or 560 long pass.

Fluorescence can be read by many platforms, such as fluorescence imager, fluorimeter, fluorescence plat readers, and laser scanner.



Assay Protocol

- Prepare serial dilutions of a protein or peptide samples in water (e.g. a 4-fold serial dilutions ranging from 0.655mg/mL to 40 ng/mL. See *Table 1*). The standard curve should be prepared ideally using the same protein or peptide species and buffer as the sample peptide to be quantified. Alternatively, use BSA standard (#UP36859A).
- Prepare a working solution of EpicoccoStab reagent by mixing dye and Bicarbonate buffer in a ratio of 1:9. (See *Table 2* for appropriate dilutions).
- To a known volume of sample or standard add an equal volume of EpicoccoStab reagent working solution. A blank should be prepared by adding equal volumes of working reagent and buffer. For a microtiter plate (100µL) assay, add 50µL of sample and 50µL of EpicoccoStab working solution.
- Incubate in the dark for 60 minutes at room temperature.
- Using a fluorescence microtiter plate reader, measure fluorescence using a 540±10 nm excitation filter and a 630±10nm emission filter (see above for equipment/detection). The fluorescence signal is stable for at least 6 hours. Elevated temperatures do not adversely affect signal.
- Subtract background fluorescence of the control from all other values and plot fluorescence over protein and peptide quantity (log₁₀ fluorescence vs log₁₀ protein and peptide quantity).
- Use the standard curve to determine the concentration of protein and peptide in the unknown sample. Note that a linear fit is normally used, but a larger dynamic range can be achieved with an exponential fit.
- Interfering compounds should be below the indicated concentrations (*Table 3*).
Note: Ideally the same buffer should be used for the protein and peptide standard and the sample of unknown concentration.

Sample and reagent preparation tables

Table 1. Preparation of a 4-fold serial dilution

Tube N°.	Water /Buffer	Protein and Peptide Standard (1 volume)	Final protein and peptide Concentration
1	-	655 360ng/mL	655 360ng/mL
2	3 volumes	163 840ng/mL	163 840ng/mL
3	3 volumes	40 960ng/mL	40 960ng/mL
4	3 volumes	10 240ng/mL	10 240ng/mL
5	3 volumes	2 560ng/mL	2 560ng/mL
6	3 volumes	640ng/mL	640ng/mL
7	3 volumes	160ng/mL	160ng/mL
8	3 volumes	40ng/mL	40ng/mL

Table 2. Preparation of FluoProbes® Protein & Peptide Quantification working solution

Number of Assays			Volume to prepare		
with 1mL cuvette	with 100µL in 96 well plate	with 20µL in 384 well plate	Dye (µL)	Bicarbonate Buffer (µL)	Total Volume (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	2500	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

Interfering Compounds

Acceptable

Maximum

Limit is

defined as the difference in fluorescence intensity exceeding $\pm 25\%$ relative to control in any of four protein concentrations tested.

The assay is effective for difficult proteins that usually show high protein-to-protein variations. However, EpicoccoStab Protein & Peptide Quantification is not accurate for heme-containing proteins.

Table 3. Maximum limits of various interfering compounds

Compound	Maximum limit
SDS	0.05%
CHAPS	0.01% +
NP40	0.005%
Thiourea	500mM +
Urea	500mM
Triton X100	0.005% v/v
Tween 20	0.01% v/v
Dithiothreitol	1.5mM +
Tributylphosphine	5µM
Iodoacetamide	50mM
Calcium chloride	500µM
TRIS-HCl	500µM
NH ₄ CO ₃	500µM
HCl	500µM +
TFA	0.005%
Formic acid	0.01%
ACN	0.5%

Additional Information

Applications

Thanks to main features, EpicoccoStab Protein & Peptide Quantification Kit is particularly suited to proteomic applications where samples are complex and difficult to analyse with conventional methods, and where downstream use of peptides / proteins is important.

EpicoccoStab dye interaction with peptides and proteins is reversible rendering the method amenable to mass spectrometry, N-term sequencing and to functional assays. As no protein is lost, it is ideal to quantify small samples before peptide mass fingerprinting and expression analysis by mass spectrometry. Because EpicoccoStab does not precipitate or denature peptides, peptide and protein samples can be used in subsequent assays.

EpicoccoStab Protein & Peptide Quantification Kit is robust to substances that inhibit most protein assays (see interfering compounds) making it ideal for 2-D and 1-D electrophoresis, IEF gels and anywhere accurate quantification of protein is needed.

Mass spectrometry and HPLC

With EpicoccoStab Protein & Peptide Quantification kit, peptide samples used for monitoring proteolytic digestion can be analysed directly by mass spectrometry without any pre-treatment. Mass spectrometric peptide mass fingerprinting (PMF) of the same peptide sample used for quantification was assessed by MALDI-TOF MS*. EpicoccoStab Protein & Peptide Quantification provides the best accuracy of Amino Acid Assay for peptide quantification at a fraction of the right cost. It is ideal for high throughput analysis.

N-term sequencing and to functional assays

1-D and 2-D Gel Electrophoresis

EpicoccoStab Peptide Quantification kit is robust to interfering compounds such as DNA and solubilisation reagents used in 1-D and 2-DGE sample preparation

Biochemistry – purification, labeling

EpicoccoStab Protein & Peptide Quantification kit is robust to many interfering compounds used in biochemistry works as solubilisation reagents and reducers. It suits for measuring complex mixtures, peptides from proteolytic digestions as well most pure peptides to monitor purifications and labeling procedures. It eliminates additional steps, such as precipitation to remove buffers or detergents that may alter protein content. Absorbance / fluorescence of the label should however not overlap the dye spectra.

*Applications notes (PMF, Glycoproteins, tryptic digestion,...) are available on inquire.

References

1. **Bell, P.J.L. and Karuso, P.** Epicocconone, a novel fluorescent compound from the fungus *Epicoccum nigrum*. *Journal of American Chemical Society*. 125:9304 (2003)
2. **Coghlan, D. R. et al.** Mechanism of reversible fluorescent staining of protein with epicocconone. *Organic Letters*. **7**:2401-240 (2005)
3. **Galas L. et al.** "Probe, Sample, and Instrument (PSI)": The Hat-Trick for Fluorescence Live Cell Imaging, *Chemosensors* **6**:40 (2018)

Related Products

- 0.1M Sodium Bicarbonate-Sodium Carbonate Buffer, pH 9.0 (#R16490)
- BSA standard (#UP36859A).
- Colorimetric protein μ BC Assay (#UP75860A) [IS](#)
- Protein Gel Stain 100X, RED Epicocconone based (#FP-1K9520) [IS](#)
- 3Dye Cy2/3/5 fluor Labeling Pack (#EV0870)

Other Information

For any question, please ask FluoProbes®: Hotline: +33 4 70 03 73 06
Catalog size quantities and prices may be found at <http://www.interchim.com>.

Legal

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