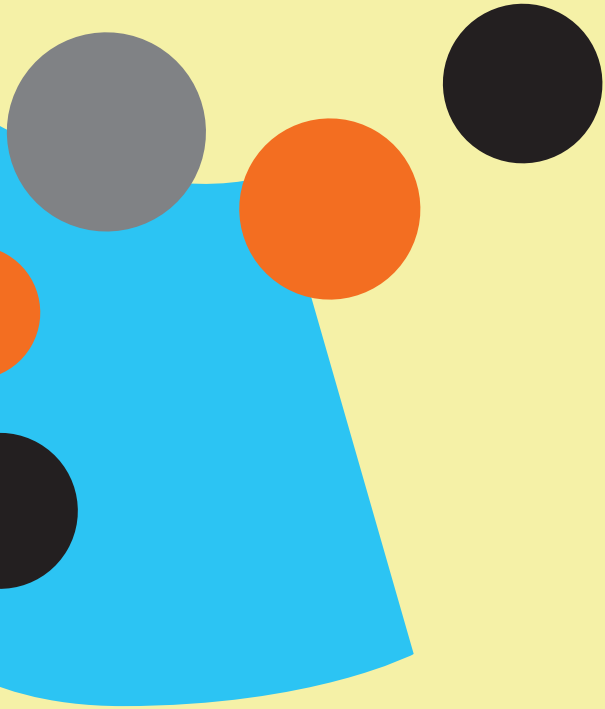
LavaPep™
Peptide and Protein Quantification Kit



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LavaPep and Protein peptide quantification kit for up to 2000 assays

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LavaPep Protein & Peptide quantification kit.

LavaPep is a fluorescence-based protein and peptide quantification kit. Peptide quantification is prerequisite in many areas of proteomics and peptidomics. Colorimetric assays (ninhydrin, Lowry, BCA) often lack the sensitivity to accurately quantify peptides. AAA is expensive, often inconvenient, and sensitive to many interfering compounds.
LavaPep depends on a small, naturally-occurring fluorescent compound[1] that reversibly binds to lysine, arginine, and histidine residues in peptides, and it responds to hydrophobic environments to yield an intensely red-fluorescent product[2]. This mechanism allows highly sensitive quantification of protein and peptides over a wide linear dynamic range. Uniquely, LavaPep tracelessly quantifies protein and peptides, enabling the same sample to be used for peptide quantification and downstream analyses (e.g. MS, HPLC and Edman chemistry).

Quick Facts

Storage

Part A at –15 °C to –30 °C in the original brown bottle to protect from light.
Part B at 3-6 °C.

Disposal

LavaPep is an environmentally safe solution and requires no special disposal procedures.

Detection

Optimum excitation wavelengths: 405, 500 nm. Suitable light sources include green (e.g. 543, 532 nm) blue (e.g. 488 nm); violet (e.g. 405 nm) or UVA.
Emission wavelength: The maximum emission is at 610 nm, irrespective of the excitation source. Suitable filters include the 610 nm band pass or 560 long pass.

Features

LavaPep Kit:

- has a linear dynamic range between 100 ng/mL and 160 µg/mL.
- is sensitive to < 100 ng/mL.
- is compatible with downstream analyses such as MS and HPLC.
- is simple and requires no heating steps.
- is more robust than other peptide assays and more cost effective than AAA.
- Is suitable for measuring peptides from proteolytic digestions and most pure peptides.

Safe Handling and Disposal

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. LavaPep part A is a dilute DMSO/acetonitrile solution of a natural organic dye. Part B 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.
- LavaPep 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.
- LavaPep is suitable for quantification of most peptides but individual standard curves are required for each peptide.

- Prepare fresh fluorophore solution in each assay (see protocol below).
- Use microtitre plates that are suited for fluorescence measurements.

Reagents and Equipment

Kit components

Component A consists of a 10× concentrate (10 mL) of the fluorophore used for peptide quantification.

Component B comprises 10× concentrate (10 mL) of bicarbonate buffer.

Reagents and Equipment not provided

- Reverse Osmosis water
- Microcentrifuge tubes 1.5 mL
- 96-well plate (black, flat-bottom)
- Fluorescence plate reader, with an excitation filter (540±10 nm) and an emission filter (630±10 nm)

Protocol

- Prepare serial dilution of a peptide in water (e.g. a 4-fold serial dilution ranging from 0.655 mg/ml to 40 ng/mL. See Table 1 for preparing a 4-fold serial dilution). The peptide standard curve should be prepared using the same protein or peptide species and buffer as the sample protein / peptide to be quantified.
- Prepare a working solution of LavaPep reagent by mixing water, Part A and Part B in a ratio of 8:1:1. (See Table 2 for appropriate dilutions).
- To a known volume of sample or standard add an equal volume of LavaPep working solution. A blank should be prepared by adding equal volumes of working reagent and buffer/water. For a microtitre plate (100 µL) assay, add 50 µL of sample and 50 µL of LavaPep working solution.

- Incubate in the dark for 60 minutes at room temperature.
- Using a fluorescence microtitre plate reader, measure fluorescence using a 540±10 nm excitation filter and a 630±10 nm emission filter.
- Subtract background fluorescence of the control from all other values and plot fluorescence over peptide quantity (log10 fluorescence vs log10 peptide quantity).
- Use the standard curve to determine the concentration of 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 at, or below, the indicated concentrations (Table 3). Ideally the same buffer should be used for the standard and the sample of unknown concentration.

Table 1. Preparation of a 4-fold serial dilution

Tube No.	Water /Buffer	Peptide Standard (1 volume)	Final peptide Concentration
1	–	655360 ng/mL	655360 ng/mL
2	3 volumes	655360 ng/mL	163840 ng/mL
3	3 volumes	163840 ng/mL	40960 ng/mL
4	3 volumes	40960 ng/mL	10240 ng/mL
5	3 volumes	10240 ng/mL	2560 ng/mL
6	3 volumes	2560 ng/mL	640 ng/mL
7	3 volumes	640 ng/mL	160 ng/mL
8	3 volumes	160 ng/mL	40 ng/mL

Table 2. Preparation of LavaPep working solution

Number of Assays			Volume			
1 mL cuvette	100 µL in 96 well plate	20 µL in 384 well plate	Water (µL)	Part A (µL)	Part B (µL)	Total Volume (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

Interfering Compounds

Acceptable Maximum Limit is defined as the difference in fluorescence intensity exceeding ± 25% relative to control in any of four protein concentrations tested (0, 10, 100 and 1000 µg/mL).

Table 3. Maximum limits of various interfering compounds

Compound	Maximum limit
SDS	0.05%
CHAPS	0.01%
NP40	0.005%
thiourea	500 mM
urea	500 mM
triton X 100	0.005% v/v
tween 20	0.01% v/v
dithiothreitol	1.5 mM
tributylphosphine	5 µM
methyl methanethiol sulfonate	1 mM
triethylammonium bicarbonate	2.5 mM
Tris(2-carboxyethyl)phosphine	500 µM
iodoacetamide	50 mM
calcium chloride	500 µM
tris-HCl	500 µM
NH_4CO_3	500 µM
HCl	500 µM
TFA	0.005%
Formic acid	0.01%
acetonitrile	0.5%

Mass spectrometry

With LavaPep, peptide samples used for monitoring proteolytic digestion can be analysed directly by mass spectrometry without any pre-treatment.

Related Products

gelcompany offers a range of related products including total protein gel and blot stains, a protein quantification kit, a protease digestion monitoring kit, and a live cell imaging reagent. For details of these and our new products visit our website at www.gelcompany.com

Legal

References

1. Bell, P.J.L. and Karuso, P. (2003) Epicocconone, a novel fluorescent compound from the fungus Epicoccum nigrum. Journal of American Chemical Society. 125, 9304.

2. Coghlan, D. R., Mackintosh, J. & Karuso, P. (2005). Mechanism of reversible fluorescent staining of protein with epicocconone. Organic Letters. 7, 2401-240.

LavaPep Protocol Version 1.2 March 2009