INSTRUCTIONS

Pierce Quantitative Fluorometric Peptide Assay, 500 Assays

23290 2593.0

Number	Description
23290	**Pierce Quantitative Fluorometric Peptide Assay**, sufficient reagents for 500 microplate assays

Kit Contents:

Fluorometric Peptide Assay Buffer, 50mL

Fluorometric Peptide Assay Reagent, 4 × 2.5mL, peptide labeling reagent in acetonitrile

Peptide Digest Assay Standard (1mg/mL), 1.5mL, tryptic digest of a protein standard, in 50mM ammonium bicarbonate and 0.05% sodium azide

Storage: Upon receipt store at 4°C. Product shipped with ice packs.

Introduction

The Thermo Scientific™ Pierce™ Quantitative Fluorometric Peptide Assay is a sensitive, mix and read microplate assay for measuring the concentration of synthetic peptides and peptide mixtures from tryptic digests. Quantitation is achieved using an amine-reactive fluorescent detection reagent that specifically labels the N-terminus of peptides. The fluorescently labeled peptides are then detected at Ex 390nm/Em 475nm. This sensitive assay only requires 10µL of sample; produces a linear response with increasing peptide concentrations over a broad working range (5-1000µg/mL); and results in a stable final fluorescence, allowing for multiple samples to be simultaneously assayed.

Important Product Information

- This assay is based on the reaction between a labeling reagent and the N-terminal primary amine in the peptide(s); therefore, samples must be free of amine-containing buffers (e.g., Tris-based buffer and/or amino acids).
- This assay cannot be used to measure the concentration of peptides labeled with amine-reactive isobaric tags, such as Thermo Scientific™ TMTsixplex™ and Thermo Scientific™ TMT10plex™ Label Reagents. However, the assay can be used to monitor the Thermo Scientific™ TMT™ Labeling Process to confirm complete modification. For samples that are N-terminal labeled and/or those containing free amines, use the Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay (Product No. 23275).
- The standard provided with this assay is optimized to produce concentration measurements that are consistent with the average results from tryptic digests of proteins or cell lysates. This assay and standard can also be used for individual synthetic peptides or digests using other proteases; however, differences in individual peptide sequence, peptide length, peptide solubility, and peptide secondary structure may affect results. As an alternative, for the individual synthetic peptide, this standard can be used to determine the molar concentration of peptide and convert to concentration in µg/mL.
- This assay is optimized to be performed in a 96-well microplate with a sample volume of ≤ 10µL and total assay volume of 100µL. If a larger assay volume is required (e.g., for cuvette-based measurements), then maintain the Sample:Assay Buffer:Assay Reagent ratio at 1:7:2 (v/v) to avoid shifts in pH or dilution of the assay reagent.
- Do NOT premix Fluorometric Peptide Assay Buffer and Fluorometric Peptide Assay Reagent.

Additional Material Required

- Ultrapure water
- Sealing tape (e.g., Sealing Tape for 96-Well Plates, Product No. 15036)
- Fluorescent-compatible, 96-well microplate (96-well black plate, Product No. 88378)
- Fluorescence plate reader compatible with Ex 390nm/Em 475nm (e.g., Thermo Scientific™ VarioSkan™ Flash Multimode Reader)

Preparation of Standards

- For samples that contain complex mixtures of peptides generated from a tryptic digestion, use the procedure in Table 1 to prepare a dilution series of the Peptide Digest Assay Standard to generate a standard curve (Fluorescence vs. µg/mL, Figure 1). Dilute the Peptide Digest Assay Standard into clean vials, preferably using the same diluent as the sample(s). The method in Table 1 will provide sufficient volume to run a 9-point standard curve (from 0-1000µg/mL) in triplicate.
- For the individual synthetic peptide sample with a molecular weight of 900-1500Da, use the same procedure as above for a complex mixture of peptides.
- For the individual synthetic peptide sample with a molecular weight **outside** of 900-1500Da, use the last column in Table 1 to generate a standard curve (Fluorescence vs. µM, Figure 2), measuring samples in µM and converting µM to µg/mL using the following formula:

  Concentration (µg/mL) = Peptide molecular weight (Da) × Concentration (µM)/1000

  Note: Peptide Standard concentrations are provided in µg/mL or µM, depending on the sample type.

Table 1. Preparation of diluted Peptide Digest Assay Standards.

Centrifuge tubes	Volume of Diluent (µL)	Volume of Digest (µL)	Final Standard Concentration for Peptide Mixture (Figure 1) (µg/mL)	Final Standard Concentration for Individual Peptides (Figure 2) (µM)
A	0	150 of Stock	1000	841.0
B	75	75 of Vial A	500	420.5
C	75	75 of Vial B dilution	250	210.3
D	75	75 of Vial C dilution	125	105.1
E	75	75 of Vial D dilution	62.5	52.6
F	75	75 of Vial E dilution	31.3	26.3
G	75	75 of Vial F dilution	15.6	13.2
H	75	75 of Vial G dilution	7.8	6.6
Blank	75	0	0	0

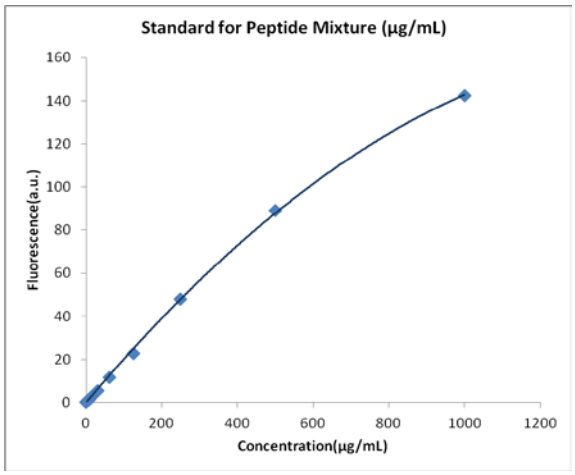

Figure 1. Example standard curve for peptide mixture.

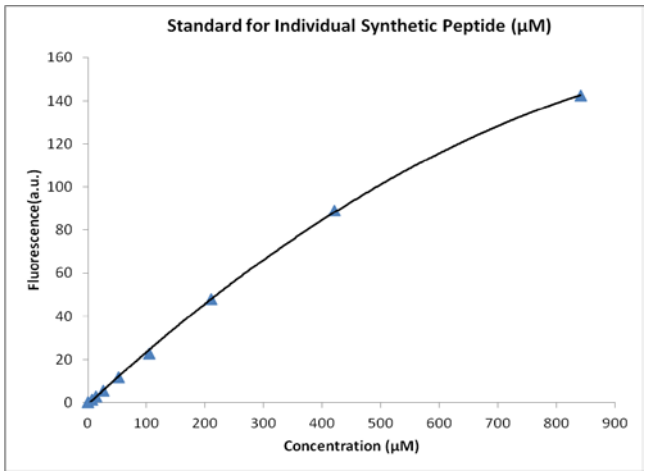

Figure 2. Example standard curve for individual synthetic peptide.

Microplate Procedure

- Equilibrate kit components to room temperature before opening and loading. Use sealing tape (Product No. 15036) to cover plate for incubation or storage. Remove sealing tape before making fluorescence measurements.
- Ensure that individual peptides or digests are fully solubilized before use.

1. Prepare assay standards and samples.
2. Pipette 10µL of each standard or sample replicate into one well of the fluorescence-compatible microplate.
3. Add 70µL of Fluorometric Peptide Assay Buffer to each well or load this buffer using a multichannel pipette and reservoir.
4. Add 20µL of Fluorometric Peptide Assay Reagent to each well.
5. Incubate at room temperature for 5 minutes.

   Note: Incubation times > 5 minutes may be used; however, the reaction is completed within 5 minutes and minimal change in signal will occur with extended incubation of up to 30 minutes.
6. Measure the fluorescence using Ex/Em at 390nm/475nm.
7. Use the standard curve to determine the peptide concentration of each unknown sample.

   Note: Assay produces a near-linear response across the recommended concentration ranges. However, if using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) fit can also be used as an alternative to a linear fit for a standard of 0-1000µg/mL.

Troubleshooting

Problem	Possible Cause	Solution
Fluorescence is too high (over maximal)	Fluorescence Gain setting was too high	Adjust gain in plate reader
Reading from unknown samples is not within the standard curve	Peptide concentration was too high or too low	Dilute or concentrate sample
	Poor peptide solubility	Dissolve peptides in an organic solvent such as DMSO or 50% acetonitrile before assaying
	Incomplete protein digestion	Non-digested protein produces a relatively minimal reaction with this assay. Verify sample digestion by SDS-PAGE
	Sample contained interfering substances	Dialyze or dilute sample
		Remove interfering substances from sample (Table 2)

Interfering and Compatible Substances

- Amino acid- and Tris-containing buffers are NOT compatible with this assay.
- Peptides labeled with amine-reactive Thermo Scientific™ TMT™ Tags are NOT compatible with this assay.
- For most substances used in tryptic digestion, the maximum compatible concentrations are listed in Table 2. Substances were compatible at the indicated concentration if the change of sample concentration caused by the presence of the substance was ≤ 15%.

Table 2. Compatible substance concentrations in the Thermo Scientific Pierce Quantitative Fluorometric Peptide Assay.

Substance	Compatible Concentration
Acetone	25%
Acetonitrile	50%
Ammonium acetate	100mM
Ammonium bicarbonate	50mM
DMSO	50%
DTT(dithiothreitol)	10mM
EDTA	25mM
Formic acid	0.1%
Guanidine	1M
Iodoacetamide	100mM
Methanol	25%
SDS	1%
Sodium azide	1%
TCEP	10mM
TEA acetate	100mM
TEA bicarbonate	100mM
Trifluoroacetic acid	0.2%
Urea	1M

Note: It is possible to have a substance additive effect even though a single component may be present at a concentration below its listed compatibility. A sample buffer containing a combination of substances could potentially interfere with the assay. Combinations of reagents have not been examined in detail.

Related Thermo Scientific Products

15075	**Reagent Reservoirs,** 200/pkg
15036	**Sealing Tape for 96-Well Plates,** 100/pkg
23275	**Pierce Quantitative Colorimetric Peptide Assay,** 500 assays
23295	**Peptide Digest Assay Standard (1mg/mL),** 1.5mL
84868	**Pierce™ High pH Reversed-Phase Peptide Fractionation Kit**
88378	**96-Well Black Plates,** 25/pkg

Current product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

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