INSTRUCTIONS

Pierce Quantitative Colorimetric Peptide Assay, 500 Assays

23275 2592.0

Number	Description
23275	**Pierce Quantitative Colorimetric Peptide Assay**, sufficient reagents for 500 microplate assays

Kit Contents:

Colorimetric Peptide Assay Reagent A, 50mL

Colorimetric Peptide Assay Reagent B, 2 × 25mL

Colorimetric Peptide Assay Reagent C, 2mL

Peptide Digest Assay Standard (1mg/mL), 1.5mL

Storage: Upon receipt store at 4ºC. Product shipped on ice packs.

Note: If Colorimetric Peptide Assay Reagent A, B or C precipitates upon shipping in cold weather or during long-term storage, dissolve precipitates by gently warming and stirring solution. Discard any kit reagent that shows discoloration or evidence of microbial contamination.

Introduction

The Thermo Scientific™ Pierce™ Quantitative Colorimetric Peptide Assay provides modified BCA reagents along with a proprietary chelator and uses the biuret reduction of Cu^{+2} to Cu^{+1} to analyze peptide mixtures. In the reaction, copper is first reduced by the amide backbone of peptides under alkaline conditions (the biuret reaction), followed by the proprietary chelator coupling with the reduced copper to form a bright red complex with absorbance of 480nm. The signal produced from this reaction is three- to four-fold more sensitive than the Thermo Scientific™ Micro BCA™ Protein Assay Kit for peptide analysis. The kit also contains a high quality peptide digest reference standard to generate linear standard curves and calibration controls. This colorimetric peptide assay requires a small amount of sample (20µL) and has a working peptide concentration range of 25-1,000µg/mL. The increased sensitivity, low sample assay volume, and included reference standard are essential for accurate, robust measurement of peptide digest samples, specifically for mass spectrometry applications.

Important Product Information

- 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 is not recommended for individual peptide quantitation.** The peptide hydrophobicity; length; and specific amino acid content, such as the presence of cysteine, tryptophan and tyrosine residues are all known to influence the rate of copper reduction, which in turn affect color formation at 480nm.
- Since the assay is based on copper reduction by amide bonds, full-length protein or partially digested protein will produce a colorimetric assay response that can interfere with results. Ensure complete peptide digestion before analysis. If the sample contains significant amounts of undigested protein, use the Thermo Scientific™ Pierce™ Quantitative Fluorometric Peptide Assay (Product No. 23290).
- Reducing and chelating reagents such as DTT and EDTA will interfere with the assay.
- Amine-reactive labeling reagents (e.g., Thermo Scientific™ TMT™ Isobaric Labeling Reagents or biotinylation reagents) are compatible with this assay.
- This method is not a true end-point method; that is, the copper continues to be reduced and color develops over time. However, the rate of color development is sufficiently slow at room temperature, allowing large numbers of samples to be assayed together with minimal error based on the normal timing of reagent additions.

- This assay is optimized to be performed in a 96-well microplate with a sample volume of ≤ 20µL and total assay volume of 200µL. If a larger assay volume is desired, e.g., for cuvette-based measurements, use a Sample:Assay Working Reagent ratio of 1:33 (e.g., use 30µL of sample and 970µL of working reagent to have a 1mL total solution volume). If larger volumes of sample are used, the number of assays per kit will be less than 500.

Additional Material Required

- Ultrapure water
- Sealing tape (e.g., Sealing Tape for 96-Well Plates, Product No. 15036)
- 96-well microplate (e.g., Thermo Scientific™ Pierce™ 96-Well Plates, Product No. 15041)
- 96-well microplate reader compatible with absorbance measurement at 480nm (e.g., Thermo Scientific™ VarioSkan™ Flash Multimode Reader)

Preparation of Standards

Use the procedure in Table 1 to prepare a dilution series of the Peptide Digest Assay Standard to generate a standard curve. Dilute the Peptide Digest Assay Standard into clean vials, preferably using the same diluent as the sample(s). This will provide sufficient volume to run an 8-point standard curve (from 0-1000µg/mL) in triplicate.

Table 1. Preparation of diluted Peptide Digest Assay Standards.

Centrifuge tubes	Volume of Water (µL)	Volume of Digest (µL)	Final Concentration of Digest (µg/mL)
A	0	150 of Stock	1000
B	75	75 of Vial A	500
C	75	75 of Vial B dilution	250
D	75	75 of Vial C dilution	125
E	75	75 of Vial D dilution	62.5
F	75	75 of Vial E dilution	31.3
G	75	75 of Vial F dilution	15.6
Blank		Use water for blank	0

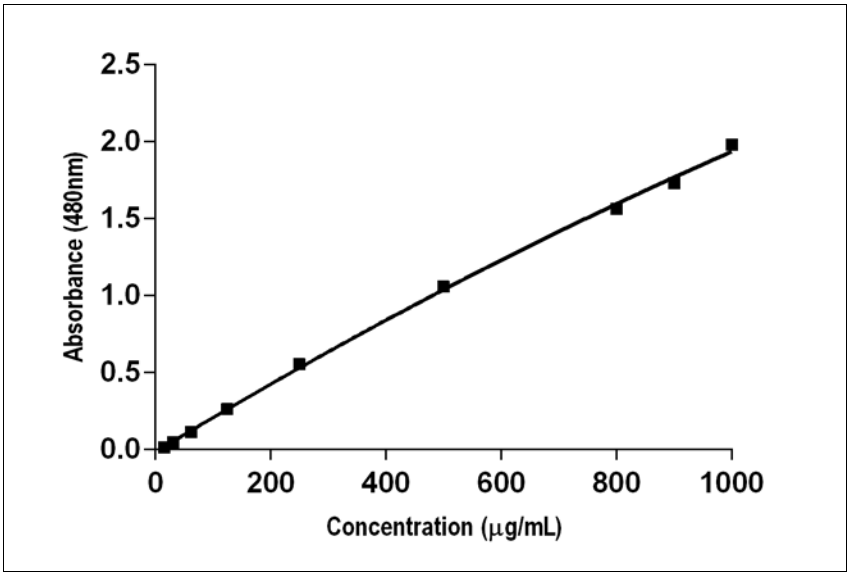

Figure 1. Example standard curve.

Preparation of Working Reagent

1. Use the following formula to determine the total volume of Working Reagent (WR) required:

   [(# of standards) + (# of unknowns)] × (# of replicates) × (0.18mL of WR per sample) = total volume WR required

   Example: For an 8-point standard curve and 5 unknowns in triplicate:

   (8 standards + 5 unknowns) × (3 replicates) × (0.18mL) = 7.02mL of WR required

   Note: For the above example, make at least 7.5mL of WR to ensure sufficient reagent for use.

2. Prepare WR by mixing:

   - 50 parts of Colorimetric Peptide Assay Reagent A
   - 48 parts of Colorimetric Peptide Assay Reagent B
   - 2 parts of Colorimetric Peptide Assay Reagent C

   For the above example, combine 3.75mL of Peptide Assay Reagent A, 3.6mL of Peptide Assay Reagent B and 0.15mL of Peptide Assay Reagent C.

   Note: After preparation, the WR can be kept at room temperature for ≤ 30 minutes.

Procedure

1. Prepare samples and standards.
2. Pipette 20µL of each standard or unknown sample replicate into a well of a 96-well microplate.
3. Add 180µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds to 1 minute.
4. Cover plate and incubate at 22°C (room temperature) for 30 minutes or 37°C for 15 minutes.

   Note: Incubation time can be increased to greater than 15 minutes if there is no sufficient color development within 15 minutes at 37°C. Increased incubation time or temperature will increase the assay response; however, prolonged incubation at elevated temperatures may reduce the linearity of the assay response. Follow instrument instructions for determining sample concentrations based on the standard curve. Alternatively, prepare a standard curve by plotting the average blank-corrected 480nm measurement for each standard versus its concentration in µg/mL. Use the standard curve to determine the peptide concentration of each unknown sample based on the average blank-corrected absorbance value of the samples.

   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) can also be used as an alternative to a linear fit.

Troubleshooting

Problem	Possible Cause	Solution
No color in wells	Sample contained a copper chelating agent	Clean-up sample on a C18 column
		Dilute sample
Blank absorbance measures correctly, but standards and samples show less color than expected	Strong acid or alkaline buffer altered WR pH	Dilute sample
		Neutralize pH
	Color measured at the wrong wavelength	Measure the absorbance at 480nm
Sample colors appear darker than expected	Peptide concentration was too high	Dilute sample
	Tryptic-digested sample contained undigested protein	Confirm digestion efficiency by SDS-PAGE. Optimize digestion protocol
	Sample contained a reducing agent such as DTT or EDTA	Clean-up sample on a C18 column
Sample colors appear very light, but standards measure correctly	Peptide sample precipitated	Dissolve the sample in 50% acetonitrile and perform assay at 37ºC for 30 minutes
	Peptide bound to tubing	
	Peptide had significant secondary structure	

Compatible Substances

- Reducing and chelating reagents such as DTT and EDTA will interfere with the assay. Clean samples to remove residual interfering reagents before assaying.
- Other than reducing agents and chelators, the Pierce Quantitative Colorimetric Peptide Assay is compatible with a wide range of standard laboratory chemicals. The maximum concentrations tested and shown to be compatible for many substances are listed in Table 2. Substances were compatible at the indicated concentration if the error in the concentration estimation caused by the presence of the substance was ≤ 15%.

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

Substance	Compatible Concentration
Acetone	50%
Acetonitrile	50%
Ammonium bicarbonate	50mM
DMSO	50%
DTT(dithiothreitol)	Not compatible
EDTA	5mM
Formic acid	0.5%
Guanidine	0.25M
Iodoacetamide	1M
Methanol	25%
SDS	1%
Sodium azide	1%
TCEP	Not compatible
TEA acetate	5mM
TEA bicarbonate	5mM
Trifluoroacetic acid	0.5%
Tris	100mM
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

15041 **Pierce 96-Well Plates,** 100/pkg

15075 **Reagent Reservoirs,** 200/pkg

15036 **Sealing Tape for 96-Well Plates,** 100/pkg

23290 **Pierce Quantitative Fluorometric Peptide Assay,** 500 assays

28904 **Trifluoroacetic acid,** 10 × 1mL

51101 **Acetonitrile,** 1L

87782 **Pierce™ C18 Tips, 100µL bed column,** 96/pkg

84840 **Mass Spec Sample Prep Kit for Cultured Cells,** 20-rxn kit

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