

Product Information

Protease Colorimetric Detection Kit

Product Code **PC0100**

Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Protease Colorimetric Detection Kit provides a complete set of reagents to detect and quantify protease activity. For convenience the kit utilizes a simple assay that can be performed in either cuvette or multiwell plate format. The assay method uses a casein substrate. As the casein is cleaved by the protease, trichloroacetic acid (TCA) soluble peptides are generated. These peptides contain tyrosine and tryptophan residues that react with the Folin & Ciocalteu's (F-C) Reagent to produce a color change. The F-C Reagent will also react with peptides containing cystine, cysteine, and histidine residues, but to a lesser extent. The amino acids reduce the tungstate and/or molybdate in the F-C Reagent, thereby generating one or more compounds with a characteristic blue color that can be colorimetrically quantitated at 660 nm.

This kit has been tested to detect a wide variety of proteases, including serine, cysteine, metallo, and aspartic proteases. Furthermore, this kit has been optimized to detect and quantify a diverse range of proteases found in physiological applications, and quantifies most non-specific proteases.

This kit will detect 0.1 unit/ml (approximately 2 µg) of trypsin with a 10 minute incubation time. This sensitivity may be increased by modifications, such as increased incubation time. If even higher sensitivity is required, the Protease Fluorescent Detection Kit (Product Code PF0100) is recommended.

Components

Sufficient reagents are provided to perform 200 one ml assays.

Protease Detection Substrate: Casein 20 x 10 mg
 supplied as a powder
 (Product Code P 9996)

2.0 N Folin & Ciocalteu's Phenol Reagent 10 ml
 (Product Code F 9252)

500 mM Sodium Carbonate Solution 125 ml
 (Product Code S 5444)

Enzyme Diluent 125 ml
 10 mM Tris acetate with 5 mM calcium
 acetate, pH 7.5
 (Product Code E 9155)

1.1 mM Tyrosine Standard Solution 1 ml
 (Product Code T 2950)

Protease from *Bacillus polymyxa* 1 g
 (Product Code P 6141)

0.6 N Trichloroacetic Acid (TCA) 5 ml
 (Product Code T 0199)

Equipment Required but Not Provided

- Pipettes
- Cuvettes or 96 well plates
- Microcentrifuge tubes
- Microcentrifuge
- Spectrophotometer suitable for measuring absorption at 660 nm

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Protease Detection Substrate - The substrate should be freshly prepared. Resuspend one 10 mg vial of casein powder (Product Code P 9996) in 1.5 ml of deionized water. This is sufficient for 10 one ml assays.

Folin & Ciocalteu's (F-C) Reagent - Mix 1 part of the 2.0 N Folin & Ciocalteu's Phenol Reagent (Product Code F 9252) with 3 parts of deionized water. For example, to prepare 4 ml, add 1 ml of the 2.0 N Folin & Ciocalteu's Phenol Reagent to 3 ml of deionized water.

TCA Working Solution - Prepare a 110 mM working solution by mixing 920 μ l of the 0.6 N TCA Solution (Product Code T 0199) with 4.08 ml of deionized water, for a total volume of 5.0 ml.

Protease Control – Use the Protease from *Bacillus polymyxa* (Product Code P 6141) to prepare a solution containing 0.1–0.2 unit per ml in the Enzyme Diluent (Product Code E 9155). The activity of the Protease from *Bacillus polymyxa* is approximately 1 unit per mg of solid. The Protease Control can be used to confirm the assay is performing properly. For the assay of a different, specific protease, it is recommended to prepare a control solution containing the specific protease in the appropriate incubation buffer.

Protease Sample - In order to quantify the protease activity in the sample, prepare an enzyme solution containing 0.1–0.2 unit per ml in the provided Enzyme Diluent. If the activity is unknown, several dilutions may be required to determine the optimal concentration range.

Storage/Stability

The kit and its components are stable as supplied, for at least 2 years if stored at 2–8 °C.

When the kit is received, the 2.0 N Folin & Ciocalteu's Phenol Reagent (Product Code F 9252) can be stored at room temperature. The reagent should be bright yellow in color. Discard any reagent that has acquired a green tint.

It is recommended that working solutions be freshly prepared.

Procedure

This kit has been optimized to detect a diverse range of proteases found in physiological applications. It is suitable for detection of serine, cysteine, metallo, and aspartic proteases; however, modifications may be required to detect some specific proteases. Modifications to the procedure may include pH adjustments, the addition of metal ions, or a reformulation of the incubation buffer. The researcher must determine the optimal procedure conditions for the protease specific to their application. Note that this procedure can be scaled up or down according to the requirements of the instruments being used.

1. To a microcentrifuge tube, add 130 μ l of Protease Detection Substrate and 25 μ l of the Protease Sample or Control. For protease samples with high protease activity, a dilution may be required.
2. To prepare the Blank sample, add 130 μ l of the Protease Detection Substrate and 25 μ l of Enzyme Diluent to a microcentrifuge tube.
3. Mix and incubate at 37 °C for exactly 10 minutes.
4. After incubation, add 130 μ l of the TCA Working Solution to each microcentrifuge tube.
5. Mix and incubate at 37 °C for 20 minutes.
6. Centrifuge the tubes for 5 minutes at 10,000 x g.

Absorbance Measurements

Note: The Sodium Carbonate Solution and diluted F-C Reagent need to be added to the sample in the same order as specified in the following steps.

Cuvettes

7. Being careful not to disturb any pellet that may have formed, pipette 250 μ l of the supernatant (step 6) into a separate fresh microcentrifuge tube or other suitable container.
8. Add 625 μ l of the Sodium Carbonate Solution (Product Code S 5444) and 125 μ l of the diluted F-C Reagent to each microcentrifuge tube.
9. Mix and incubate at 37 °C for 30 minutes.
10. Cool to room temperature.
11. Transfer 1 ml to a suitable cuvette.
12. Mix and record the absorbance at 660 nm.

Multiwell Plates

7. Pipette 50 μ l of the supernatant (step 6), 125 μ l of the Sodium Carbonate Solution (Product Code S 5444), and 25 μ l of the diluted F-C Reagent into each well of a 96 well plate.
8. Seal or cover the plate to avoid evaporation.
9. Incubate at 37 °C for 30 minutes.
10. Remove the cover and record the absorbance at 660 nm.

Tyrosine Standard Curve

A standard curve is generated using the Tyrosine Standard Solution (Product Code T 2950), thereby facilitating quantitation of the protease activity.

1. Generate the standard curve by pipetting reagents (see Table 1) into microcentrifuge tubes or other suitable containers.

Table 1.
Reaction Scheme for Tyrosine Standard Curve

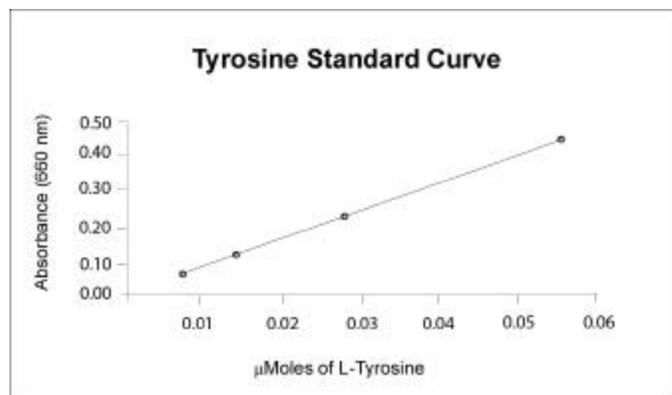
Reagent	Standard Blank	Standard 1	Standard 2	Standard 3	Standard 4
Tyrosine Standard	0.0 µl	5.0 µl	10.0 µl	25.0 µl	50.0 µl
Water	250 µl	245 µl	240 µl	225 µl	200 µl
Sodium Carbonate	625 µl	625 µl	625 µl	625 µl	625 µl
Diluted F-C Reagent	125 µl	125 µl	125 µl	125 µl	125 µl
µmoles of Tyrosine	0	0.0055	0.0110	0.0275	0.055

- Mix and incubate at 37 °C for 30 minutes.
- Transfer 1 ml to a suitable cuvette or 200 µl to each well of a 96 well plate.
- Record the absorbance at 660 nm.
- Subtract the absorbance of the Standard Blank from the absorbance of each Standard.

$$?A_{660 \text{ Standard}} = A_{660 \text{ Standard}} - A_{660 \text{ Standard Blank}}$$

- Plot the $?A_{660}$ versus µmoles of tyrosine (see Figure 1).

Figure 1.
Typical Standard Curve generated using the Tyrosine Standard Solution.



Protease Activity Calculations

The protease activity is determined using the Tyrosine Standard Curve. A standard curve should be generated each time the assay is performed.

- Subtract the absorbance of the Sample Blank from the absorbance of each Sample.

$$?A_{660 \text{ Sample}} = A_{660 \text{ Sample}} - A_{660 \text{ Sample Blank}}$$

- Determine the µmoles of tyrosine equivalents liberated using the equation derived for the Tyrosine Standard Curve.
- Determine the units of protease activity per ml of protease sample using the following equation:

$$\text{Units/ml} = \frac{\mu\text{mole of Tyr} \times \text{reaction volume}}{\text{sample vol} \times \text{reaction time} \times \text{vol assayed}}$$

µmole of Tyr = µmole of tyrosine equivalents released
 reaction volume = total volume (in ml) of assay
 sample vol = volume (in ml) of protease sample used
 reaction time = time (in minutes) of reaction incubation
 vol assayed = volume (in ml) used in colorimetric determination

Unit Definition: One unit will hydrolyze casein to produce color equivalent to 1.0 µmole (181 µg) of tyrosine per minute at pH 7.5 at 37 °C (color by Folin & Ciocalteu's Reagent).

For example, using the procedure for absorbance measurement with a cuvette and a tyrosine equivalent equal to 0.0275 µmoles, the units/ml would be determined as follows:

$$\text{Units/ml} = \frac{0.0275 \times 0.285}{0.025 \times 10 \times 0.25} = 0.125 \mu\text{moles ml}^{-1} \text{ min}^{-1}$$

References

- Anson, M.L., J. Gen. Physiol., **22**, 79–89 (1938).
- Folin, O., and Ciocalteu, V., J. Biol. Chem., **73**, 627 (1929).
- A Practical Guide to Enzymology, Suelter, C.H., John Wiley & Sons, Inc. (New York, NY: 1985).

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