PDQ™ Protease Assay

Catalog Numbers: 0201 - 0202

Product Description

The PDQ™ (Protease Determine Quick) Protease Assay is a colorimetric assay used to detect protease activity in aqueous samples. The proprietary substrate responds to a wide range of proteases including serine, metallo, aspartate and cysteine proteases such as collagenase, proteinase K, papain, pepsin, bromelin, ficin, trypsin and chymotrypsin. PDQ™ can be used with just a few simple steps to measure protease activity and requires no centrifugation. The substrate is a cross-linked matrix containing protein substrate and a dye-protein conjugate. Protease activity is detected spectrophotometrically with increasing optical density proportional to increasing enzyme activity and can detect nanogram quantities.

The PDQ™ Fluorescent Protease Assay employs the same protease lattice matrix as the Colorimetric PDQ™ Protease Assay. Fluorescein Isothiocyanate (FITC) is incorporated into the substrate as an indicator of enzymatic digestion of the substrate, allowing for detection of sub-nanogram quantities of protease activity in aqueous samples. The fluorescent-based substrate responds to the same broad spectrum of proteases as the Colorimetric PDQ™ Assay. Protease activity is detected fluorophotometrically with increasing fluorescence proportional to increasing enzyme activity.

Each kit is supplied with a trypsin solution for generating standard curves (BAEE equivalent units). PDQ™ is supplied in 48 ready-to-use plastic vials.

Product Specifications

Unit Size	48 vials of 200µL of pre-made matrix
Control	Trypsin supplied as control: 0.5mL of 0.7mg/mL (1420 BAEE units/mg) in reaction buffer (10mM Tris-Cl, pH 8.0).
Storage	4°C
Stability	3 months

Reorder Information

Catalog Number	Product
0201	PDQ™ Protease Assay
0202	PDQ™ Fluorescent Protease Assay

Materials Required but not Provided

0.2N NaOH
Spectrophotometer for reading absorbance at 450nm (standard assays)
Fluorimeter for measuring 485nm excitation and 535nm emission (for fluorescent assays only)

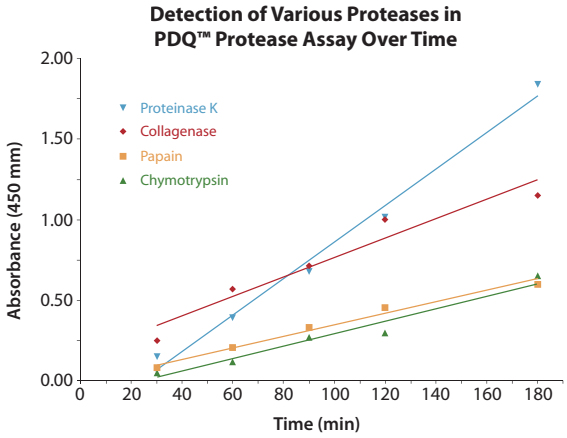

Figure 1. Time-course degradation of the matrix by papain, chymotrypsin, proteinase K, and collagenase. The reactions were incubated at 37°C and 0.2 N NaOH added to duplicate vials to stop the reaction at the indicated times. The absorbance at 450 nm was measured by pouring the reaction mixture into spectrophotometric cuvettes.

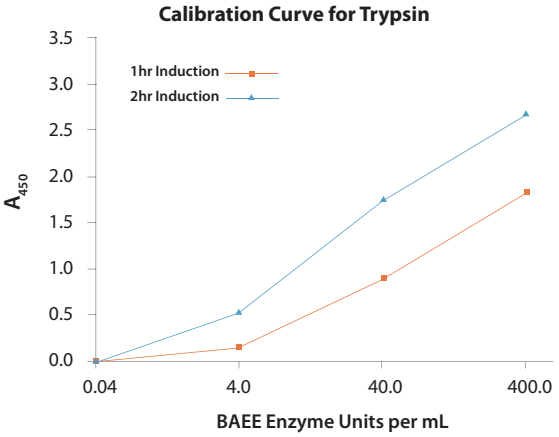

Figure 2. The above graph shows absorbance levels of the PDQ™ Colorimetric Assay as a function of enzyme concentration. The diagram shows the incremental dilutions of trypsin in 10mM Tris-Cl using 400, 40, 4.0, and 0.4 BAEE Units/mL of protease activity, with a control "blank" which contains the Tris buffer alone.

See full technical information at www.athenaes.com

Material Safety Data

FOR RESEARCH USE ONLY. NOT INTENDED OR APPROVED FOR HUMAN, DIAGNOSTICS OR VETERINARY USE. Do not ingest, swallow or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. For complete safety information see full Material Safety Data Sheet.

Instructions for Use

1. Allow the vials to come to room temperature before running the assay. Pour off storage solution.

2. To construct a standard curve, prepare a ten-fold dilution series of the control protease. This control solution should contain the protease for which the test is being run (e.g., use proteinase K in the control dilution series if this is the protease being tested for). Trypsin is supplied as a general protease control. The trypsin can be used in a ten-fold dilution series; e.g., 400 –> 0.4 BAEE units/ml (280 - 0.28 g/ml). Any buffer can be used with the PDQ™ matrix. (See Technical Considerations). The standard trypsin reaction buffer is 10mM Tris-Cl, pH 8.0.

3. Add 0.5 ml of each control solution to duplicate vials.

4. Prepare test samples containing putative protease activity in the same buffer as the control protease. Add 0.5 ml of the test sample to duplicate vials.

5. Replace snap caps onto vials.

6. Incubate vials at 37°C for 1 hr. (A shorter or longer incubation time may be required. See Technical Considerations).

7. Add 0.5 ml 0.2N NaOH to each vial to stop the reaction and amplify color. Either gently swirl or tap the vial or replace the cap and invert the vial once to mix contents. (Add 1.5ml 0.1N NaOH if assaying the contents in standard cuvettes).

8. Transfer the contents (being careful not to disturb the semi-solid matrix) to semi-micro or standard cuvettes.

9. For spectrophotometric assays, record absorbance at 450 nm. For fluorimetric assays, record the fluorescence for FITC settings (485 nm excitation/ 535 nm emission).

10. Average the results from duplicate vials and use the control samples to generate a standard curve, plotting concentration (or amount) of control protease vs. A450 or fluorescence. Compare test sample results against the standard curve produced by the control protease dilution series to obtain semi-quantitative protease activity data.

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Technical Considerations

1. Buffers that can be used to prepare test samples may range in pH from 4 to 10.

2. The amount of time required for incubation can be varied to optimize the assay. Up to 24 hr incubations are possible which will increase the detection limits of the assay. Alternatively, for applications with high amounts of enzyme, 15 min may suffice. The exact conditions should be determined empirically.

3. As with any assay, it is necessary to run a standard curve with control protease every time the assay is performed with "unknown" protease samples.

4. When using the vials, the stopped reaction contents must be transferred to a cuvette to measure the absorbance. The transfer of the contents away from the PDQ™ matrix should be done as soon as possible after the addition of NaOH, since the NaOH will degrade the matrix over time. However, the contents can be left on the matrix for up to one hour before transfer without compromising the accuracy of the test.

5. Once the stopped reaction contents are transferred away from matrix, they can be left for up to 24 hrs covered (to keep out light) at room temperature before measuring the absorbance without loss of signal.

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