Product Data Sheet

No. 10

PDQ™ Protease Assay

Catalog Numbers: 0201 and 0202

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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 **QuantaWell PDQ™ Protease Assay** employs the same protease substrate as the Colorimetric PDQ™ Protease Assay. Fluorescein is incorporated into the substrate and the substrate coated onto the surface of a QuantaWell2.0™ microtiter dish (Cat. No. 0206). QuantaWell2.0™ plates contain metal nano-particles that enhance fluorescent signals via a plasmonic effect. This allows for detection of picogram 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 by measuring the residual amount of fluorescense remaining on the coated plate or by quantifying the released fluorescence in the solution.

Product Specifications

Catalog Number	0201 Colorimetric PDQ™ Protease Assay
Kit 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

Catalog Number	0202 QuantaWell PDQ™ Protease Assay
Kit Size	96-well Strip Well Plate
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	2 years

Materials Required but not Provided

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

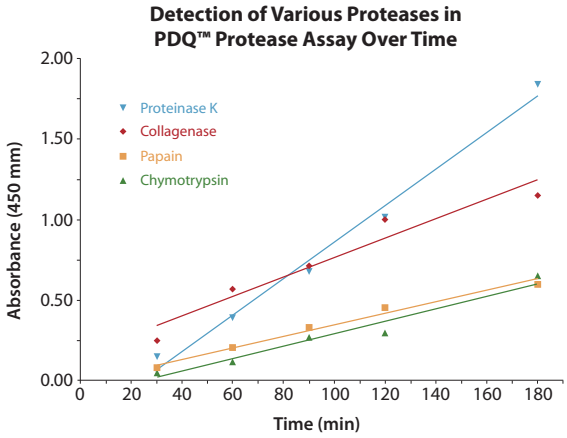

Figure 1. Time-course degradation of the matrix by papain, chymotrypsin, proteinase K, and collagenase.

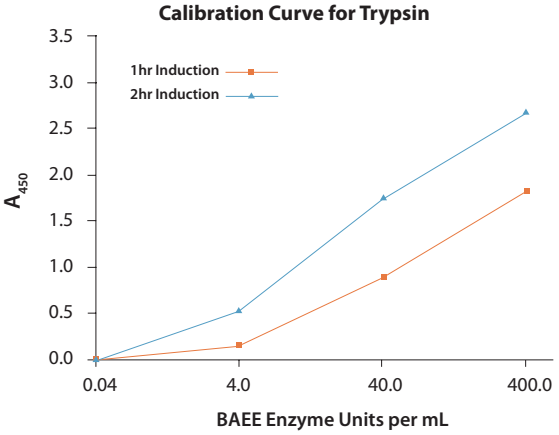

Figure 2. Calibrator cuvres generated using the PDQ™ Colorimetric Assay. Trypsin was at 400, 40, 4.0, and 0.4 BAEE Units/mL incubated for 1 or 2 hours.

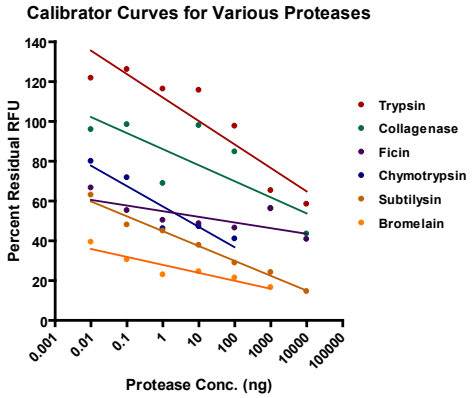

Figure 3. Calibrator cuvres generated using the QunataWell PDQ™ Assay. The amont of the different proteases per reaction is shown. Reactions were incubated for 1 hour and the residual fluorescence measured.

Instructions for Use

***Colorimetric Assay*:**

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. Trypsin is supplied as a general protease control. The trypsin can be used in a ten-fold dilution series; e.g., 400 to 0.4 BAEE units/ml (280 - 0.28 µg/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 37ºC for 1 h. (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 the vial or replace the cap and invert the vial once to mix contents.
8. Transfer the contents (being careful not to disturb the semi-solid matrix) to a standard 1 ml cuvette. (Transfer 0.2 ml of liquid if using a microplate reader.)
9. Record the absorbance at 450 nm.
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. Compare test sample results against the standard curve to obtain the amount of protease activity.

***QuantaWell Fluorometric Assay*:**

1. Remove foil from the desired number of strips and wash the wells three times with ~0.4 ml of reaction buffer.
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. Trypsin is supplied as a general protease control. The trypsin can be used in a ten-fold dilution series; e.g., 40 to 0.04 BAEE units/ml (28 - 0.028 µg/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. Prepare test samples containing putative protease activity in the same buffer as the control protease.
4. Add 0.1 ml of each control solution to duplicate wells.
5. Add 0.1 ml of the test sample(s) to duplicate wells.
6. Incubate the reactions at 37ºC for 1 h in a humid chamber or cover the wells with sealing tap. (A shorter or longer incubation time may be required. See Technical Considerations.)
7. Remove the liquid from each well. (Reserve the liquid if desired. The fluorescense in the liquid represents releasted substrate.)
8. Wash each well three times with ~0.4 ml of reaction bufffer. Remove any residual liquid by smacking the strips upside down on a paper towel.
9. Record the fluorescense, 485ex and 535em, remaining in each well. This is the residual fluorescense.
10. Average the residual RFU from the duplicate wells and use the control samples to generate a standard curve, plotting concentration (or amount) of control protease vs. residual RFU. Compare test sample results against the standard curve to obtain the amount of protease activity.

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.
6. The QuantaWell PDQ™ Protease Assay is particularly useful if the test samples are opaque. The sample matrix, once removed from the wells, does not interfere with the fluorescence measrement.
7. To use the Colorimetric Assay for opaque samples, run the reaction as described. Remove the reaction liquid and then back digest the remaining substrate matrix with Trypsin, Subtilisin or Proteanase K. The signal generated from the back digestion is inversely proportional to the amount of enzyme activity.

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.