CREATIVE BIOMART®
Assay Kit

Protease Assay Kit

Product Information

Cat.No.

Kit-2416

Product Overview

The Protease Assay Kit enables easy, fast and reproducible quantitation of protease activity. The homogeneous assay is performed in a microplate and requires no strong acids or separation steps. TPCK trypsin is provided as a general protease standard and may be used for secondary calibration of other specific protease standards. The assay method uses succinylated casein and trinitrobenzenesulfonic acid (TNBSA).1-3 Succinylated casein is native casein that has been treated with succinic anhydride to block primary amines on the surface of the protein. In the presence of protease, the succinylated casein is cleaved at peptide bonds, thereby exposing primary amines (predominantly a-amines). TNBSA reacts with these exposed primary amines to produce an orange-yellow product whose intensity may be measured at 450nm. The increase in color relative to sample without succinylated casein is a measure of protease activity in the sample.1 Because TNBSA produces color when it reacts with any primary amine, it is critical that blanks for each sample be included in the assay to correct for the effect of amines (general protein content) in the protease sample. Amine-containing buffers such as Tris will cause high background, although correction for small amounts of Tris (< 50mM) in the sample can be made using the blank. In practice, when the concentration of amines in the buffer or sample is high, the sample must be diluted so that its blank (no succinylated casein) absorbance at 450nm in the assay is less than 0.500. The assay is compatible with up to 1mM DTT in the protease sample; however, the background (blank) absorbance reading will be increased.

Size

250 tests

Kit Components

Succinylated Casein, 5 × 10mg, supplied as lyophilized salt-free powder
TNBSA (2,4,6-trinitrobenzene sulfonic acid), 5% (w/v) in methanol, 2mL
TPCK Trypsin, 50mg; specific activity: > 10,350 BAEE units/mg of protein

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Borate Buffer Pack, yields 50mM borate, pH 8.5 after reconstitution with 500mL water
Caution: TNBSA is a skin sensitizer and stain. Avoid skin contact with the TNBSA solution

Materials Required but Not Supplied

• Pipettors capable of accurately dispensing 50μL, 100μL, and 5mL. An 8-channel pipettor is very convenient and wellsuited for large numbers of samples.
• Microplate(s). Use standard clear 96-well plates or 8-well strip plates.
• Optional: Microtube Racked System. This system of 1.1mL tubes in a 96-well format allows for convenient manipulation of samples to be dispensed with a multi-channel pipettor.
• Plate reader capable of measuring absorbance at 450nm.
Note: Other wavelengths between 400-500nm can be used but will result in decreased sensitivity.

Preparation

Assay Buffer: Prepare by dissolving contents of the Borate Buffer Pack in 500mL distilled water. After reconstitution, the composition of this buffer will be 50mM borate, pH 8.5.
Note: The assay can be performed using other buffers, provided they are free of primary amines (e.g., Tris). Optimal pH is 8.0 or higher.
Succinylated Casein Solution: Dissolve 1 vial (10mg) of lyophilized Succinylated Casein in 5mL of Assay Buffer. Let the vial stand for 5 minutes after addition of buffer, then gently swirl the vial to fully dissolve the protein. Five milliliters of succinylated casein solution will be sufficient to assay 48 samples in a 96-well microplate.
Note: It is normal for the Succinylated Casein Solution to be slightly opalescent.
Trypsin Stock Solution: Dissolve the lyophilized TPCK Trypsin in 1mL of ultrapure water or Assay Buffer to make a 50mg/mL stock solution. Prepare small (10-50μL) aliquots of this stock and store at -80°C. Thaw a new aliquot each time an assay is performed; do not refreeze a thawed aliquot.
Trypsin Standard: Thaw one aliquot of Trypsin Stock Solution and dilute to 0.5mg/mL in Assay Buffer. Serially dilute this solution to yield 6-8 standards that can be used to construct a standard curve for the assay. A series of 2-fold dilutions may not span the full range of detectable protease activity; consider making a series of 5- or 10-fold dilutions (see Additional Information Section).
Note: Trypsin serves as a general standard for comparison of overall protease activity among different samples. However, optimum conditions for activity of other proteases are likely to be

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different than for trypsin. To accurately measure activity of a specific protease, use known amounts of that protease to prepare the standard curve. If standard curves of both trypsin and the protease of interest are prepared, a calibration may be established between the two proteases.
TNBSA Working Solution: Prepare by adding 100μL of the supplied TNBSA stock to 14.9mL of Assay Buffer.
Caution: TNBSA is a skin sensitizer and stain. Wear gloves. A paper towel dipped in concentrated detergent can be used to wipe up spills.

Assay Protocol

Note: The assay method requires use of a control blank for each unknown and standard sample. Each blank contains buffer and protease sample (unknown or standard) but no Succinylated Casein Solution. When assayed in parallel to the samples, these blanks control for color development caused by amines in the protease sample, including those cause by self-cleavage.
1. Add 100μL Succinylated Casein Solution to one set of microplate wells. Add 100μL Assay Buffer to a duplicate set of wells to serve as blanks.
2. Add 50μL of each unknown or standard sample to both Succinylated Casein wells and corresponding blank wells.
3. Incubate plate for 20 minutes at room temperature (RT).
Note: Warmer incubation temperatures (up to 37°C) can also be used; in general, this will increase protease activity.
4. Add 50μL TNBSA Working Solution to each well.
5. Incubate plate for 20 minutes at RT.
6. Measure absorbances of wells in a plate reader set to 450nm. Other wavelengths between 400-500nm can be used but will result in decreased sensitivity.
7. For each well calculate the change in absorbance at 450nm (ΔA450) by subtracting the A450 of the blank from that of the corresponding Casein well. This ΔA450 is the absorbance generated by the proteolytic activity of the protease.
Note: Samples or buffers containing high concentrations of proteins and other amine-containing compounds may result in blanks that have A450 values greater than 0.500. In such cases, assessment of proteolytic activity by the Pierce Method will be inaccurate. Dilute samples in Assay Buffer so that they have blank A450 values < 0.500.

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8. Plot a standard curve (ΔA450 against protease standard concentration) and use to assess relative protease activity of the unknown sample.

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