CREATIVE BIOMART®
Assay Kit

Asparaginase Activity Assay Kit

Product Information

Cat

Kit-0107

Common Name

Asparaginase

Cat.No.

Kit-0107

Product Overview

Simple procedure; takes 40 minutes
Fast, reliable and convenient
Kit can be used to detect Asparaginase enzyme's antineoplastic effects, based on the inability of cancer cells (unlike healthy cells) to synthesize asparagine.

Description

Asparaginase (EC 3.5.1.1) is a homotetramer that catalyzes the hydrolysis of asparagine to aspartic acid and ammonia and exhibits about a 2-4% activity on glutamine and 5% on D-asparagine. Asparaginase does not occur naturally in humans but is found in bacteria, plants and many animals (e.g. guinea pigs). The enzyme has been used to reduce acrylamide, a suspected carcinogen, produced in fried starchy food products and to treat acute lymphoblastic leukemia (ALL) and some other hematopoietic neoplasms (e.g. multiple myeloma). Metabolization of asparagine prevents acrylamide formation in fried foods (Maillard reaction). The enzyme's antineoplastic effects are based on the inability of cancer cells (unlike healthy cells) to synthesize asparagine. However, the enzyme is not without some antigenicity and toxicity so it is very important to measure its activity in biological samples or monitor its activity during therapy. We provides a simple, direct and automation-ready procedure for measuring asparaginase activity in biological samples. In the assay, Asparaginase hydrolyzes asparagine to generate aspartic acid, which can be detected colorimetrically (λ = 570 nm) or fluorescently (Ex/Em = 535/590 nm) using a coupled enzymatic reaction.

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Usage

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.

Size

100 assays

Kit Components

Asparaginase Assay Buffer
OxiRed Probe (in DMSO)
Substrate Mix
Aspartate Enzyme Mix
Conversion Mix
Positive Control
Aspartate Standard (100 mM)

Detection method Colorimetric
Fluorescence Plate Reader

Compatible Sample Types

Bacteria
Food Products
Tissue Lysates
Cell Lysates

Preparation

Ready to use as supplied. Warm the probe (usually 2-5 min in 37°C bath) to melt the DMSO and mix well prior to use. Store at -20°C, protect from light and moisture. Use within two months.
Substrate Mix: Reconstitute with 0.5 ml ddH2O. Store at -20°C. Avoid multiple freeze/thaw cycles. Use within two months.
Aspartate Enzyme Mix, Conversion Mix: Reconstitute each with 220 µl Assay Buffer. Pipette up and down to completely dissolve. Aliquot and store at -20°C. Avoid freeze/thaw cycles. Use within two months.

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Positive Control: Reconstitute with 100 µl Assay Buffer. Pipette up and down to completely dissolve. Aliquot and store at -20°C. Avoid freeze/thaw cycles. Use within two months.
Aspartate Standard: Warm to room temperature before use. Store at -20°C.

Assay Protocol

1. Standard Curve Preparations:
Colorimetric assay: Dilute the Aspartate Standard to 1 nmol/µl by adding 10 µl of the Standard to 990 µl of Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of standards wells. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2,4, 6, 8, and 10 nmol/well of the Aspartate Standard.
Fluorometric assay: For samples with low asparaginase activity, fluorometric assay is desirable. Further dilute the 1 nmol/µl standard 10 more folds to generate 0, 0.2,0.4, 0.6, 0.8, 1 nmol/well of the Aspartate Standard. Fluorometric assays are 10 times more sensitive than the colorimetric assay.
2. Sample and Positive Control Preparations:
Prepare samples to 50µl/well with Assay Buffer in a 96-well plate. Serum can be directly added into sample wells. Tissues or cells can be extracted with 4 volume of the Assay Buffer, centrifuge to remove insoluble materials. For the positive control, add 5 µl positive control solution to wells, adjust volume to 50 µl/well with Assay Buffer. Aspartate, Oxaloacetate, and Pyruvate in samples will generate background. We suggest using several different doses of your sample to ensure the readings are within the linear range.
3. Reaction Mix Preparation: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 50 µl Reaction Mix containing:
40 µl Assay Buffer
4 µl Substrate Mix
2 µl Aspartate Enzyme Mix
2 µl Conversion Mix
2 µl OxiRed Probe*
*Notes: For fluorometric assay, use 0.4 µl probe per reaction to reduce fluorescence background.
4. Add 50 µl of the reaction mix to each well containing the aspartate standard, positive controls, or test samples, mix well.
5. Measure A1 at OD 570 nm (or Ex/Em = 535/590 nm for the fluorometric assay) at T1 (after □10 min) then measure A2 at OD 570 nm again at T2 after incubating the reaction at 25°C for 30 min (or

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incubate longer time if the sample activity is low), protect from light. The OD of color generated by asparaginase is ΔA570nm = A2-A1

Note: It is essential to read A1 and A2 in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A1 and A2 in the reaction linear range. From our experience, A1 should be measured after 10 minutes to decrease sample background interferences.

6. Calculation: Plot the aspartate standard Curve. Apply the ΔA570nm to the aspartate standard curve to get B nmol of aspartate (amount generated between T1 and T2 in the reaction wells).

Asparaginase Activity = Sample Dilution Factor = nmol/min/ml = mU/ml

Where: B is the generated aspartate amount from Aspartate Standard Curve (in nmol).

T1 is the time of the first reading (A1) (in min).

T2 is the time of the second reading (A2) (in min).

V is the pretreated sample volume added into the reaction well (in ml).

One unit is defined as the amount of asparaginase which generates 1.0 μmol of aspartate per minute at 25°C.