Asparaginase Activity Assay Kit

Catalog Number KA1429

100 assays

Version: 03

Intended for research use only

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Introduction

Background

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. Asparaginase Activity Assay Kit 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.

General Information

Materials Supplied

List of component

Component	Amount
Asparaginase Assay Buffer	25 ml
OxiRed Probe (in DMSO)	0.2 ml
Substrate Mix (lyophilized)	1 vial
Aspartate Enzyme Mix (lyophilized)	1 vial
Conversion Mix (lyophilized)	1 vial
Positive Control (lyophilized)	1 vial
Aspartate Standard (100 mM)	0.1 ml

Storage Instruction

Store kit at -20°C.

Precautions for Use

✓ FOR RESEARCH USE ONLY! Not to be used on humans.

Assay Protocol

Reagent Preparation

- ✓ Assay Buffer: Warm to room temperature before use. Store at 4°C.
- ✓ OxiRed Probe: 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 ddH_2O. 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.
- ✓ 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 Procedure

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 0.2 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 A_1 at O.D. 570 nm (or Ex/Em = 535/590 nm for the fluorometric assay) at T_1 (after ~ 10 min) then measure A_2 at O.D. 570 nm again at T_2 after incubating the reaction at 25°C for 30 min (or incubate longer time if the sample activity is low), protect from light. The O.D. of color generated by asparaginase is $\Delta A_{570nm} = A_2 - A_1$

   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.

Data Analysis

Calculation of Results

Plot the aspartate standard Curve. Apply the ΔA_{570nm} to the aspartate standard curve to get B nmol of aspartate (amount generated between T_1 and T_2 in the reaction wells).

$$\text{Asparaginase Activity} = \frac{B}{(T2 - T1) \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

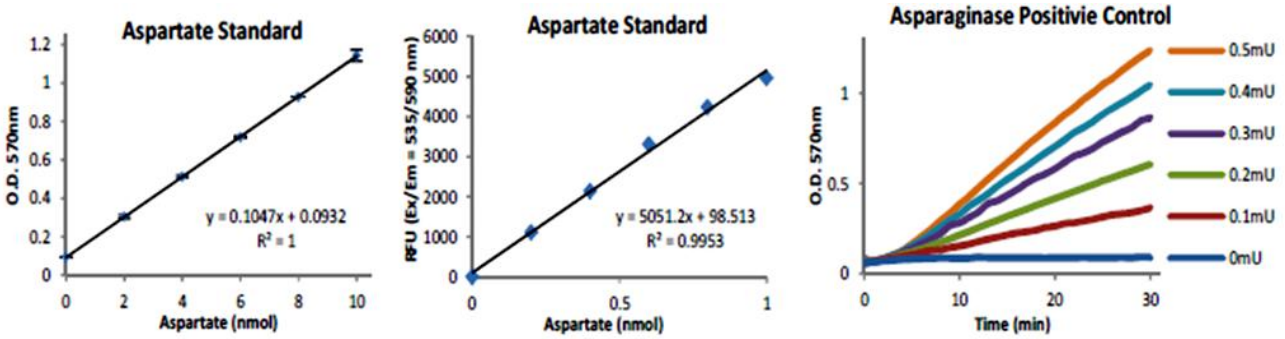
Where: B is the generated aspartate amount from Aspartate Standard Curve (in nmol).

T_1 is the time of the first reading (A_1) (in min).

T_2 is the time of the second reading (A_2) (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.



Resources

Troubleshooting

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed • Use fresh samples or store at correct temperatures until use

Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		