Asparaginase Activity Assay Kit

(Catalog #K754-100; 100 assays; Store kit at –20°C)

I. Introduction:

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. BioVision 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.

II. Kit Contents:

Components	100 Assays	Cap Code	Part Number
Asparaginase Assay Buffer	25 ml	WM	K754-100-1
OxiRed™ Probe (in DMSO)	0.2 ml	Red	K754-100-2A
Substrate Mix (lyophilized)	1 vial	Orange	K754-100-3
Aspartate Enzyme Mix (lyophilized)	1 vial	Green	K754-100-4
Conversion Mix (lyophilized)	1 vial	Purple	K754-100-5
Positive Control (lyophilized)	1 vial	Blue	K754-100-6
Aspartate Standard (100 mM)	0.1 ml	Yellow	K754-100-7

III. Reagent Preparation and Storage Conditions:

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.

IV. Pyruvate Kinase 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 A_1 at OD 570 nm (or Ex/Em = 535/590 nm for the fluorometric assay) at T_1 (after ~10 min) then measure A_2 at OD 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 OD of color generated by asparaginase is $\Delta A_{570nm} = A_2 - A_1$

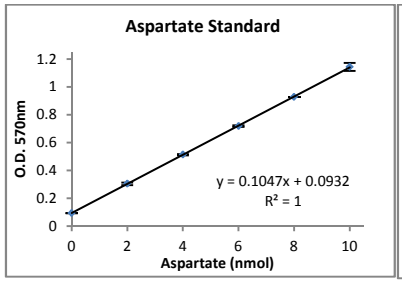
Note: It is essential to read A_1 and A_2 in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A_1 and A_2 in the reaction linear range. From our experience, A_1 should be measured after 10 minutes to decrease sample background interferences.

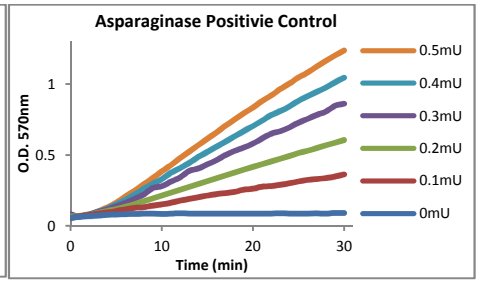
6. **Calculation:** Plot the aspartate standard Curve. Apply the ΔA_{570nm} to the aspartate standard curve to get B nmol of aspartate (amount generated between T_1 and T_2 in the reaction wells).

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





V. Related Products:

Pyruvate Assay Kit
Pyruvate Kinase Activity Assay Kit
Glutamate Assay Kit
ALT (SGPT) Activity Assay Kit
AST (SGOT) Activity Assay Kit
Glutathione Reductase Activity Assay Kit
G6PDH Activity Assay Kit
GST Fluorometric Assay Kit
Cell Proliferation Assay Kit
GDH Activity Assay Kit
Glucose Dehydrogenase Activity Assay Kit
Lactate Assay Kit
Aspartate Assay Kit
Glutamate Assay Kit
Alanine Assay Kit
Phenylalanine Assay Kit
Glutathione Peroxidase Activity Assay Kit
Uric Acid Assay Kit
Cell Proliferation Assay Kit
GST Colorimetric Assay Kit
Ammonia Assay Kits
LDH Activity Assay Kit
Alcohol Dehydrogenase Activity Assay Kit
Ethanol Assay Kit

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		

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