

RayBio® Asparaginase Activity Assay Kit

User Manual Version 1.0
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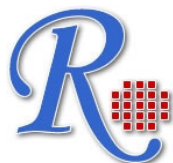
RayBio® Asparaginase Activity Assay
Kit Protocol

(Cat#: 68AT-Asp-S100)



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RayBiotech, Inc.

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TABLE OF CONTENTS

I. Introduction.....	1
II. Reagents.....	2
III. Reagent Storage & Preparation.....	2
IV. Assay Procedure	3

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 ($\lambda = 570 \text{ nm}$) or fluorescently (Ex/Em = 535/590 nm) using a coupled enzymatic reaction.

II. REAGENTS

Components	ASP-S100	Cap Code	Part Number
Asparaginase Assay Buffer	25 ml	WM	Item A
OxiRed™ Probe (in DMSO)	0.2 ml	Red	Item B
Substrate Mix (lyophilized)	1 vial	Orange	Item C
Aspartate Enzyme Mix (lyophilized)	1 vial	Green	Item D
Conversion Mix (lyophilized)	1 vial	Purple	Item E
Positive Control (lyophilized)	1 vial	Blue	Item F
Aspartate Standard (100 mM)	0.1 ml	Yellow	Item G

III. REAGENT STORAGE & 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 ddH₂O. Store at -20°C. Avoid multiple freeze/thaw cycles. Use within two months.
- Aspartate Enzyme Mix, Conversion Mix: Reconstitute each with 220 μ l 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 μ l 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.

V. Assay Procedure:

1. Standard Curve Preparations:

Colorimetric assay: Dilute the Aspartate Standard to 1 nmol/ μ l by adding 10 μ l of the Standard to 990 μ l of Assay Buffer, mix well. Add 0, 2, 4, 6, 8, 10 μ l into a series of standards wells. Adjust volume to 50 μ l/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/ μ l 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 50 μ l/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 μ l positive control solution to wells, adjust volume to 50 μ l/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 μ l Reaction Mix containing:

- 40 μ l Assay Buffer
- 4 μ l Substrate Mix
- 2 μ l Aspartate Enzyme Mix
- 2 μ l Conversion Mix
- 2 μ l OxiRed™ Probe*

***Notes:** For fluorometric assay, use 0.4 μ l probe per reaction to reduce fluorescence background.

4. Add 50 μ l 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 $\Delta A_{570\text{nm}} = A_2 - A_1$
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 $\Delta A_{570\text{nm}}$ to the aspartate standard curve to get B nmol of aspartate (amount generated between T_1 and T_2 in the reaction wells).

$$\text{Asparaginase Activity} = \frac{B}{(T_2 - T_1) \times V} \times \text{Sample Dilution Factor} = \text{nmol/min/ml} = \text{mU/ml}$$

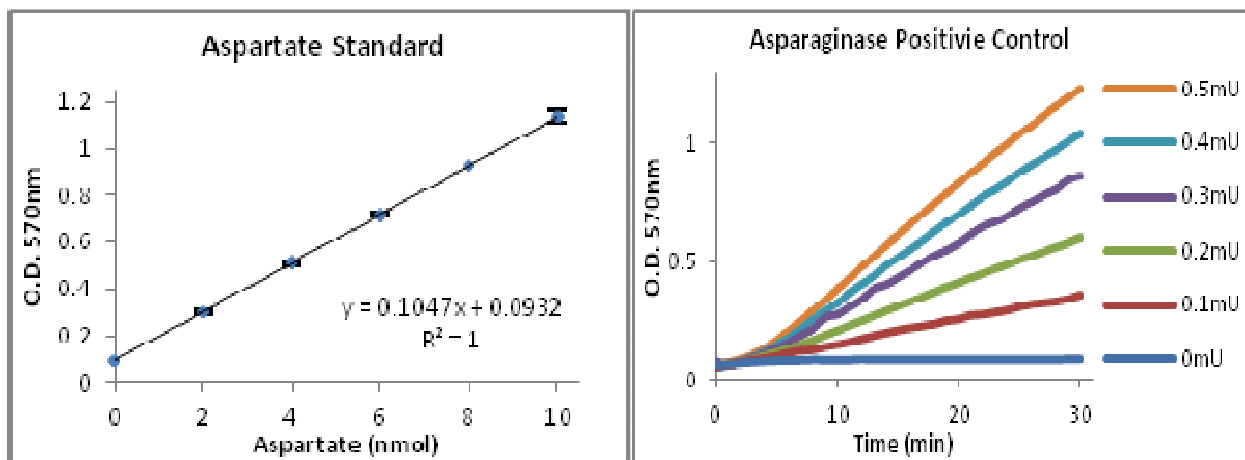
Where: B is the generated aspartate amount from Aspartate Standard Curve (in nmol).

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

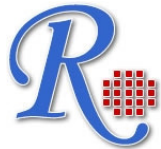
T_2 is the time of the second reading (A_2) (in min).

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.



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