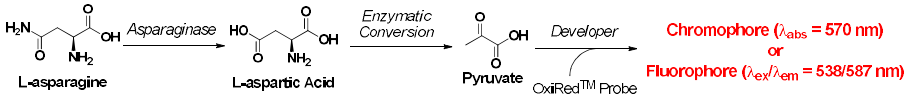
Asparaginase Activity Assay Kit (Colorimetric/Fluorometric)

(Catalog # BN00967 ; 100 Reactions; Store at -20°C)

I. Introduction:

Asparaginase (EC 3.5.1.1) is a homotetrameric enzyme that catalyzes the hydrolysis of L-asparagine to L-aspartate and ammonia. The enzyme is largely selective for L-asparagine versus other L-amino acids, but exhibits a small amount of activity (~5%) on D-asparagine. Asparaginase does not occur naturally in humans but is found in bacteria, plants and certain 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 lymphoblasts (unlike most normal cells) to synthesize L-asparagine. By destroying plasma L-asparagine, asparaginase deprives malignant lymphoblasts of the exogenous L-asparagine needed for rapid growth. However, being a bacterial enzyme, the drug does posses some antigenicity and toxicity, so it is very important to measure its activity during therapy to determine optimal dosing. Assay Genie's Asparaginase Activity Assay Kit provides a simple, direct and automation-ready procedure for measuring asparaginase activity in biological samples. In the assay, asparaginase hydrolyzes L-asparagine to generate L-aspartate, which is converted to pyruvate and subsequently reacts with a colorless probe to form a stable chromophore that is which can be detected colorimetrically (λ_{abs} = 570 nm) or fluorometrically (Ex/Em = 538/587 nm) using a series of coupled enzymatic reactions.



II. Applications:

- Estimation of Asparaginase activity in various biological samples

III. Sample Type:

- Human or animal biological fluids (plasma, serum, etc.)
- Purified asparaginase enzyme preparations

IV. Kit Contents:

Components	BN00967	Cap Code	Part Number
Asparaginase Assay Buffer	25 ml	WM	BN00967-1
GenieRed Probe (In DMSO)	200 µl	Red	BN00967-2
Substrate Mix	1 vial	Orange	BN00967-3
Aspartate Enzyme Mix	1 vial	Green	BN00967-4
Conversion Mix	1 vial	Purple	BN00967-5
Asparaginase Positive Control	1 vial	Blue	BN00967-6
Aspartate Standard (100 mM)	100 µl	Yellow	BN00967-7

V. User Supplied Reagents and Equipment:

- Multiwell fluorescence microplate reader
- Clear 96-well plates with flat bottom
- Precision multi-channel pipette

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Allow the Asparaginase Assay Buffer to warm to room temperature prior to use. Read entire protocol before performing the assay procedure.

- **GenieRed Probe:** Ready to use as supplied. Warm the probe 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 500 µl ddH2O. Store at -20°C. Avoid multiple freeze/thaw cycles. Use within two months.
- **Aspartate Enzyme Mix** and **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.
- **Asparaginase 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 (100 mM):** Warm to room temperature before use. Store at -20°C.

VII. Asparaginase Activity Assay Protocol:

1. Sample Preparation:

a. Biological fluid samples (such as plasma and serum) should be clarified by centrifugation at 10,000 x g for 5 min in order to reduce turbidity and separate insoluble material. Soft tissues (~10 mg) or cultured cells (~1 x 10^6 cells) should be rapidly homogenized on ice with 100 µl ice cold Asparaginase Assay Buffer. Centrifuge at 15,000 x g for 10 min at 4°C and transfer the supernatant to a new microfuge tube.

b. Add 2-10 µl of test sample (clarified biological fluids or tissue/cell lysates) to desired well(s) in a clear, flat bottom 96-well plate. Adjust the volume of all sample wells to 50 µl/well with Asparaginase Assay Buffer.

Notes:

- Aspartate and pyruvate present in samples will generate background. For fluorometric detection of samples expected to have high levels of aspartate or pyruvate, we recommend preparing two parallel wells for each test sample: one for determination of Asparaginase activity and one to serve as a sample background control. In our experience, there is no need to prepare corresponding sample background control wells if the assay is performed in colorimetric mode.

- Asparaginase activity can vary dramatically depending upon the sample type. We recommend performing a pilot experiment to ensure readings are within the standard curve range. Highly active samples may be diluted in Asparaginase Assay Buffer.

c. For a positive control, add 5 µl of the reconstituted Asparaginase Positive Control solution to desired well(s) and adjust the volume to 50 µl/well with Asparaginase Assay Buffer.

2. Standard Curve Preparation:

Colorimetric Assay: Dilute the Aspartate Standard to 1 nmol/µl by adding 10 µl of the Aspartate Standard (100 mM) stock 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 detection may be 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 detection is approximately 10 times more sensitive than the colorimetric assay.

3. Reaction Mix Preparation:

a. Prepare Reaction Mix according to the table below. Make a sufficient amount of the Reaction Mix to add 50 µl to each of the test sample, aspartate standard curve and positive control wells.

	Samples & Standards	Sample Background
Asparaginase Assay Buffer	40 µl	44 µl
Substrate Mix	4 µl	—
Aspartate Enzyme Mix	2 µl	2 µl
Conversion Mix	2 µl	2 µl
GenieRed Probe*	2 µl	2 µl

Note: For fluorometric assay, use 0.5 µl of GenieRed Probe per reaction to reduce fluorescence background.

b. Add 50 µl of the Reaction Mix to all test sample, standard curve and positive control wells. If applicable, for sample background control wells, add 50 µl of the Sample Background Reaction Mix (**prepared without asparaginase Substrate Mix**), bringing the final reaction volume of all wells to 100 µl.

4. Measurement: Immediately begin measuring absorbance at OD_{570} (or fluorescence at Ex/Em = 538/587 nm for the fluorometric assay) of all wells in kinetic mode at 25°C for 30-60 min (times longer than 30 min may only be needed if the sample activity is low).

Note: The Aspartate Standard curve wells may be read in endpoint mode (OD_{570} or Ex/Em = 538/587) following the kinetic reaction.

5. Calculations: For the Aspartate Standard curve, subtract the zero standard (0 nmol/well) reading from all of the standard readings, plot the background-subtracted values and calculate the slope of the standard curve. For test sample and background control wells (if applicable), choose two time points (T_1 and T_2) in the linear phase of the progress curve and calculate the change in absorbance (or fluorescence) for the time interval ($\Delta OD_{570} = A_2 - A_1$ or $\Delta F = RFU_2 - RFU_1$). It is essential to that T_1 and T_2 fall within the reaction linear phase. From our experience, the T_1 measurement should be made at $T \approx$ 5-10 minutes, as the linear phase begins roughly 5-10 mins after the initiation of the reaction. If sample background control reaction rate is significant, the sample background control rate may be subtracted from the corresponding sample rate. Apply the ΔOD_{570} to the Aspartate Standard curve to get B nmol of asparagine metabolized during the linear portion of the reaction time ($\Delta T = T_2 - T_1$).

$$\textbf{Sample Asparaginase Activity} = \frac{\boldsymbol{B}}{\Delta \boldsymbol{T} \times \boldsymbol{V}} \times \boldsymbol{D} = \textbf{nmol/min/ml = mU/ml}$$

Where: ***B*** is the amount of aspartate generated, calculated from the standard curve (in nmol)
ΔT is the linear phase reaction time: $T_2 - T_1$ (in min)
V is the volume of sample lipid extract added to the well (in ml)
D is the sample dilution factor (if applicable, D=1 for undiluted samples)

Note: One unit of asparaginase activity is defined as the amount of enzyme that generates 1.0 µmole of aspartate per minute at 25°C.

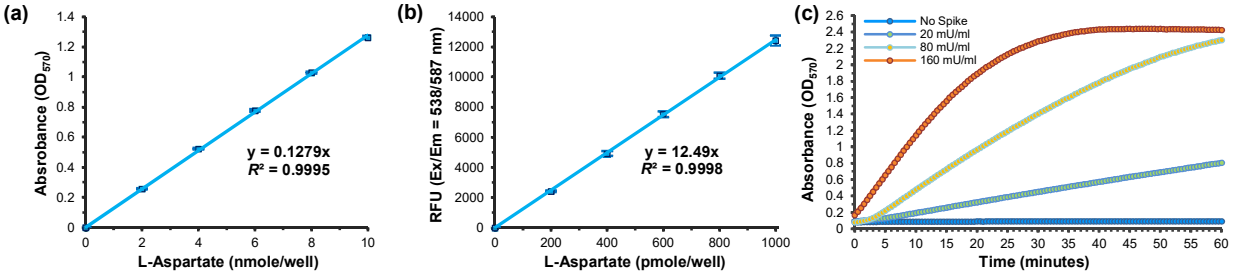


Figure: (a) Aspartate Standard curve (colorimetric detection, 0-10 nmole/well). One mole of aspartate corresponds to one mole of asparagine metabolized by asparaginase. **(b)** Aspartate Standard curve (fluorometric detection, 0-1 nmole/well). **(c)** Reaction kinetics of asparaginase activity in human serum spiked with various amounts of asparaginase from *E. coli*. Pooled normal serum (each 5 µl per well) spiked with 0, 20, 80 or 160 mU/ml of asparaginase was assayed in colorimetric mode according to the kit protocol. Calculated sample asparaginase activities were 0.28 mU/ml, 20.28 mU/ml, 76.15 mU/ml and 153.9 mU/ml, respectively. Spike recovery ranged from 95 – 102%.

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