TECHNICAL MANUAL

Asparaginase (ASNase) Activity Colorimetric Assay Kit

- **SKU CODE:** MAES0530
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add samples to wells
- Add working solution
- Measure absorbance at 340 nm

2. Intended use

This kit can be used to measure asparaginase (ASNase) activity in serum and plasma samples.

3. Detection principle

Asparaginase (ASNase) is a proteolytic enzyme that was first discovered in guinea pigs and is not naturally produced in humans. Asparaginase has been widely used in the food industry, inhibiting the production of acrylamide carcinogens by hydrolyzing asparagine. Asparaginase is also used to treat various diseases due to its unique anti-tumor properties, such as acute lymphocytic leukemia, non-Hodgkin's lymphoma, leukemia meningitis, lung cancer, ovarian cancer, and other tumor diseases.

The detection principle of this kit is as follows: asparaginase catalyzes the substrate to generate aspartic acid, and aspartic acid is hydrolyzed under the catalytic action of the enzyme. The amount of NADH consumed by the reaction is directly proportional to the activity of asparaginase. NADH is absorbed at a wavelength of 340 nm, and the activity of asparaginase can be calculated based on the change in absorbance value per unit time.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	9 mL x 1 vial	18 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 2	Coenzyme	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 3	Enzyme Reagent	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
Reagent 4	Substrate	0.1 mL x 1 vial	0.2 mL x 1 vial	-20 °C, 12 months, shading light
	UV-Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (340 nm), Incubator

Reagents:

PBS (0.01 M, pH 7.4)

6. Reagent preparation

1. Equilibrate all the reagents to 25 °C before use.
2. **Preparation of coenzyme working solution:** Dissolve one vial of coenzyme with 220 µL of double distilled water, mix well to dissolve. Store at -20 °C for a week protected from light.
3. **Preparation of enzyme working solution:** Dissolve one vial of enzyme reagent with 220 µL of double distilled water, mix well to dissolve. Store at -20 °C for a week protected from light.
4. **Preparation of working solution:** Before testing, prepare sufficient working solution according to the test wells. For example, prepare 395 µL of working solution (mix well 375 µL of buffer solution, 10 µL of coenzyme working solution and 10 µL of enzyme working solution). The prepared solution should be prepared on spot and used up within 2 hours.
5. **Preparation of substrate working solution:** Before testing, prepare sufficient substrate working solution according to the test wells. For example, prepare 250 µL of substrate working solution (mix well 20 µL of substrate and 230 µL of double distilled water). Store at -20 °C for 1 month protected from light.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly.

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

Sample type - Dilution factor

Guinea pig serum - 2-15

Guinea pig plasma - 2-15

Note: The diluent is PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

8. Operating steps

1. **Control blank well:** Add 40 μL of double distilled water into the wells. Control well/Sample blank well: Add 20 μL of double distilled water into the wells. Sample well: Add 20 μL of substrate working solution into the wells.
2. **:** Add 20 μL of substrate working solution into sample blank wells. Add 20 μL of sample into control wells and sample wells.
3. **:** Add 150 μL of working solution into each well.
4. **:** Measure the OD values of each well at 340 nm with microplate reader, as A1. Incubate at 37 °C for 10 min, measure the OD values of each well at 340 nm with microplate reader, as A2. Note: It is recommended to use kinetic detection or record the OD value once every minute for a total of 10 min. Observe whether the OD value change in the sample well within 10 min is a uniform decrease. If it is not a uniform decrease, the sample concentration needs to be adjusted.

9. Calculation

The sample:

Plasma (serum) samples:

Definition: The amount of NADH in 1 L of plasma (serum) sample that catalyzes decomposition of 1 μmol product at 37 °C for 1 min is defined as 1 unit.

ASNase activity (U/L) = ((deltaA$_{sample}$ - deltaA$_{sample}$ blank) - (deltaA$_{control}$ - deltaA$_{control}$ blank)) / (ε × d) × (V_1 / V_2) ÷ T × f × 1000

[Note]

$deltaA_{control}$: The change OD value of control well, A_1 - A_2

$deltaA_{control}$ blank: The change OD value of control blank well, A_1 - A_2

$deltaA_{sample}$: The change OD value of sample well, A_1 - A_2

$deltaA_{sample}$ blank: The change OD value of sample blank well, A_1 - A_2

ε: The molar extinction coefficient at 340 nm, 6.22 L/mmol/cm

d: Optical path, 0.6 cm

V_1: The volume of total reaction system, 190 µL

V_2: The volume of sample, 20 µL

T: Reaction time, 10 min

f: Dilution factor of sample before tested

1000: 1 mmol = 1000 µmol

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three guinea pig serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	25.00	50.00	100.00
%CV	2.2	4.7	3.1

Inter-assay Precision

Three guinea pig serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	25.00	50.00	100.00

Parameters	Sample 1	Sample 2	Sample 3
%CV	6.7	8.8	8.1

Recovery

Three samples of high concentration, middle concentration and low concentration were tested for each concentration 6 times in parallel to obtain the average recovery rate of 98.3%.

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	25.00	50.00	100.00
Observed Conc. (U/L)	24.25	50.00	98.00
Recovery rate (%)	97.0	100.0	98.0

Sensitivity

The analytical sensitivity of the assay is 1.27 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

11. Appendix II Example Analysis

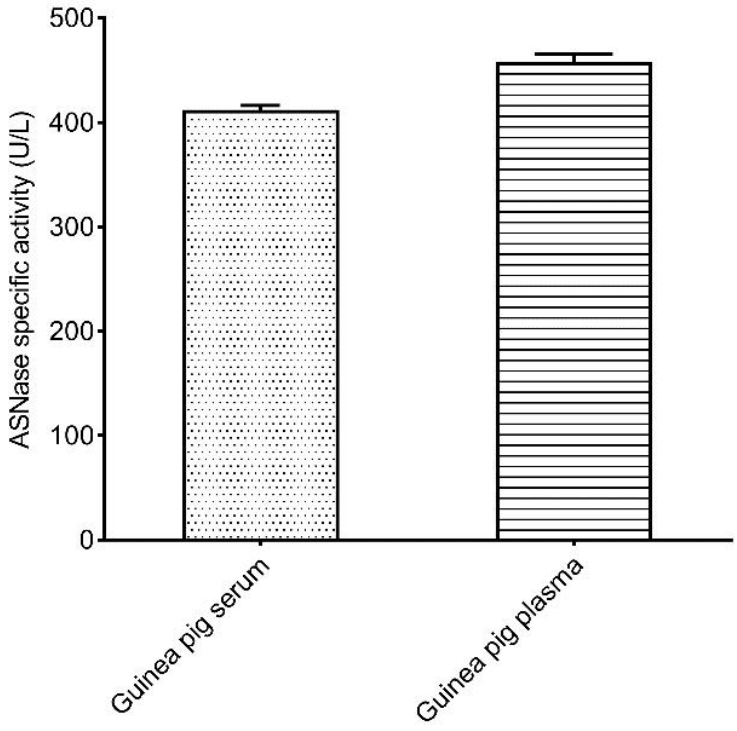
Example analysis:

Take 20 µL of guinea pig plasma (dilute by 6 times) and carry out the assay according to the operation table. The results are as follows:

The A_1 value of the sample well is 1.583, the A_2 value of the sample well is 1.262 after the reaction for 10 min, $deltaA_{sample}$ = 1.583 - 1.262 = 0.321. The A_1 value of the sample blank well is 1.627, the A_2 value of the sample blank well is 1.605 after the reaction for 10 min, $deltaA_{sample}$ blank = 1.627 - 1.605 = 0.022. The A_1 value of the control well is 1.674, the A_2 value of the control well is 1.649 after the reaction for 10 min, $deltaA_{control}$ = 1.674 - 1.649 = 0.025. The A_1 value of the control blank well is 1.622, the A_2 value of the control blank well is 1.600 after the reaction for 10 min, $deltaA_{control}$ blank = 1.622 - 1.600 = 0.022, and the calculation result is:

ASNase activity (U/L) = ((0.321 - 0.022) - (0.025 - 0.022)) ÷ (6.22 × 0.6) × (190 ÷ 20) ÷ 10 × 6 × 1000 = 452.09 U/L

Detect guinea pig serum (dilute by 3 times), guinea pig plasma (dilute by 6 times), the result is as follows:



12. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any arising problems or legal liabilities caused by using the kit for clinical diagnosis or other purposes.

2. Please read the instructions carefully and calibrate the instruments before the experiments. Please follow the instructions strictly during the experiments.

3. Protective measures must be taken by wearing lab coat and latex gloves.

4. If the concentration of the substance is not within the detection range exactly, an additional dilution or concentration should be performed on the sample.

5. It is recommended to perform a pre-test if your sample is not listed in the instruction manual.

6. The experimental results are closely related to the quality of reagents, operations, environment and other factors. Assay Genie will guarantee the quality of the kits only, and is NOT responsible for the sample consumption caused by using the

assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.