Technical Bulletin

Asparaginase Activity Assay Kit

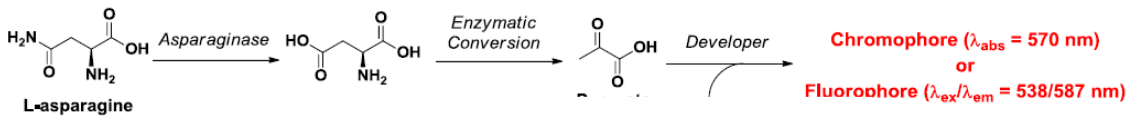
Catalogue Number MAK007

Product Description

Asparaginase is an enzyme that catalyzes the hydrolysis of asparagine to aspartate. While asparaginase is synthesized by plants, microorganisms, and some animals, it does not occur naturally in humans. While most cells have the ability to synthesize asparagine, many hematopoietic cells do not and depend on exogenous asparagine for protein synthesis. Treatment of certain hematopoietic malignancies such as acute lymphoblastic leukemia (ALL) with asparaginase depletes asparagine in the blood, resulting in cell cycle arrest and apoptosis. Asparaginase is also used in the food industry to reduce the formation of acrylamide in starchy and fried foods.

The Asparaginase Activity Assay Kit provides a simple and direct 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 which can be detected colorimetrically at 570 nm or fluorometrically (λ_{Ex} = 535 nm/ λ_{Em} = 587 nm), proportional to the aspartate generated.

The Asparaginase Activity Assay Kit is suitable for the determination of asparaginase activity in a variety of biological samples, including human or animal biological fluids such as plasma and serum, and purified asparaginase enzyme preparations.



Components

The kit is sufficient for 100 assays in 96-well plates.

- Asparaginase Assay Buffer — 25 mL
  Catalogue Number MAK007A
- Fluorescent Peroxidase Substrate, in DMSO — 0.2 mL
  Catalogue Number MAK007B
- Substrate Mix — 1 vial
  Catalogue Number MAK007C
- Aspartate Enzyme Mix — 1 vial
  Catalogue Number MAK007D
- Conversion Mix — 1 vial
  Catalogue Number MAK007E
- Asparaginase Assay Positive Control — 1 vial
  Catalogue Number MAK007F
- Aspartate Standard, 100 mM — 0.1 mL
  Catalogue Number MAK007G

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence or spectrophotometric multiwell plate reader
- 96-well flat-bottom plate. It is recommended to use black plates with clear bottoms for fluorescence assays and clear plates for colorimetric assays. Cell culture or tissue culture treated plates are not recommended.

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions.

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Asparaginase Assay Buffer: Allow buffer to come to room temperature before use. Chill an appropriate amount of Asparaginase Assay Buffer for use in Sample Preparation.

Fluorescent Peroxidase Substrate: Ready to use as supplied. Allow reagent to come to room temperature before use. Mix well by pipetting, then aliquot and store, protected from light and moisture, at –20 °C. Upon thawing, the Fluorescent Peroxidase Substrate is ready-to-use in the colorimetric assay.

Substrate Mix: Reconstitute the vial with 0.5 mL of water. Mix well by pipetting, then aliquot and store at –20 °C. Avoid freeze/thaw cycles. Use within two months of reconstitution.

Aspartate Enzyme Mix and Conversion Mix: Reconstitute each vial with 220 µL of Asparaginase Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Avoid freeze/thaw cycles. Use within two months of reconstitution.

Asparaginase Assay Positive Control: Reconstitute the vial with 100 µL of Asparaginase Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Avoid freeze/thaw cycles. Use within two months of reconstitution.

Aspartate Standard (100 mM): Ready to use as supplied. Warm to room temperature before use. Store at -20 °C.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Tissue or Cells

Rapidly homogenize tissue (~10 mg) or cells (~1 × 10^6 cells) on ice with 100 µL of ice-cold Asparaginase Assay Buffer. Centrifuge at 15,000 × g for 10 minutes at 4 °C to remove insoluble materials. Transfer the supernatant to a clean vial.

Biological fluids, Serum, or Plasma

Clarify samples by centrifuging at 10,000 × g for 5 minutes in order to reduce turbidity and separate insoluble material.

For All Samples (S)

Add 2–10 µL of Sample (clarified biological fluid or tissue/cell lysate) to desired well(s) of a clear, flat-bottom 96-well plate. Adjust the total volume in each Sample (S) well to 50 µL with Asparaginase Assay Buffer.

For unknown samples, it is recommended to test several sample volumes to make sure the sample readings are within the standard curve range.

Sample Background Control for Fluorometric Assay

Aspartate, oxaloacetate, and pyruvate in the samples will generate a background signal. Running a Sample Background Control allows for the correction of samples with high background. Use the same amount of tissue/cell lysate/biological fluid as in the Sample (S) well(s). Adjust the total volume to 50 µL/well with Asparaginase Assay Buffer.

Based on experience, a Sample Background Control is not necessary for the colorimetric assay.

Positive Control (Optional)

Add 5 µL of the reconstituted Asparaginase Assay Positive Control to designated well(s). Adjust the total volume in each well to 50 µL with Asparaginase Assay Buffer.

Colorimetric Standard Curve Preparation

Prepare a 1 mM (1 nmole/µL) Aspartate Standard by diluting 10 µL of the 100 mM (100 nmole/µL) Aspartate Standard Solution with 990 µL of Asparaginase Assay. Prepare Aspartate Standards for colorimetric assay according to Table 1. Mix well.

Table 1.
Preparation of Aspartate Standards for Colorimetric assay

Well	1 mM Aspartate Standard	Asparag-inase Assay Buffer	Aspartate(nmol/ well)
1	0 µL	50 µL	0
2	2 µL	48 µL	2
3	4 µL	46 µL	4
4	6 µL	44 µL	6
5	8 µL	42 µL	8
6	10 µL	40 µL	10

Fluorometric Standard Curve Preparation

1. Prepare a 1 mM Aspartate Standard by diluting 10 µL of the 100 mM Aspartate with 990 µL of Asparaginase Assay Buffer.
2. Further dilute the 1 mM Aspartate Standard solution by adding 10 µL to 90 µL of Asparaginase Assay Buffer, yielding a 0.1 mM Aspartate Standard working solution. Prepare Aspartate Standards for fluorometric assay according to Table 2. Mix well.

Table 2.
Preparation of Aspartate Standards for fluorometric assay

Well	0.1 mM Aspartate Standard	Asparag-inase Assay Buffer	Aspartate (nmol/ well)
1	0 µL	50 µL	0
2	2 µL	48 µL	0.2
3	4 µL	46 µL	0.4
4	6 µL	44 µL	0.6
5	8 µL	42 µL	0.8
6	10 µL	40 µL	1.0

Reaction Mix (for all wells except Sample Background Control)

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of the appropriate Reaction Mix according to Table 3. Mix well.

Table 3.
Preparation of Reaction Mix

Reagent	Colorimetric Assay Reaction Mix	Fluorometric Assay Reaction Mix
Asparaginase Assay Buffer	40 µL	41.5 µL
Substrate Mix	4 µL	4 µL
Aspartate Enzyme Mix	2 µL	2 µL
Conversion Mix	2 µL	2 µL
Fluorescent Peroxidase Substrate	2 µL	0.5 µL

2. Add 50 µL of the Reaction Mix into each well containing Standard, Positive Control and Sample(s). Mix well.

Sample Background Control Reaction Mix

1. For each Sample Background Control well, prepare 50 µL of the appropriate Reaction Mix according to Table 4. Mix well.

Table 4.
Preparation of Sample Background Control Reaction Mix

Reagent	Colorimetric Assay Reaction Mix	Fluorometric Assay Reaction Mix
Asparaginase Assay Buffer	44 µL	45.5 µL
Substrate Mix	---	---
Aspartate Enzyme Mix	2 µL	2 µL
Conversion Mix	2 µL	2 µL
Fluorescent Peroxidase Substrate	2 µL	0.5 µL

2. Add 50 µL of the Sample Background Control Reaction Mix into each well containing Sample Background Control.

Measurement

Immediately begin to measure the absorbance at 570 nm (A_{570}) or fluorescence (RFU) at λ_{Ex} = 538 nm /λ_{Em} = 587 nm in kinetic mode for 30-60 minutes at 25 °C. Note: Reaction times longer than 30 minutes may be needed if the sample activity is low.

Measure the absorbance (A_{570}) or fluorescence (RFU) in kinetic mode, and then choose any two time points (T_1 and T_2) in the linear range of the curve.

The Aspartate Standard Curve can be read in endpoint mode at the end of the incubation period.

Results

Note: Based on experience, the T_1 measurement should be taken at T = 5-10 minutes, as the linear phase of the enzymatic reaction begins ~5-10 minutes after initiation of the reaction.

1. Subtract the 0 Standard reading (A_{570} / RFU) from all readings (A_{570} / RFU).
2. Plot the Aspartate Standard Curve and calculate the slope of the curve.
3. Choose two time points (T_1 and T_2) in the linear range of the plots and calculate the change in absorbance (colorimetric or fluorometric) for the time interval for each Sample (S):

   A_{S1} = A_{570} reading at T_1
   A_{S2} = A_{570} reading at T_2
   $\Delta A_{570} = A_{S2} - A_{S1}$

   RFU_{S1} = RFU reading at T_1
   RFU_{S2} = RFU reading at T_2
   $\Delta RFU = RFU_{S2} - RFU_{S1}$

4. Apply the ΔA_{570} or ΔRFU value for each sample to the Aspartate Standard Curve to determine B nmol of Aspartate generated by the Sample during the reaction time ΔT ($\Delta T = T_2 - T_1$).
5. Calculate the Sample(S) Asparaginase activity:
   Asparaginase Activity (nmol/min/ml or mU/ml) =

   $$[B/(\Delta T \times V)] \times DF$$

where:

B = Aspartate amount from Standard Curve (nmol).

ΔT = Reaction time ($T_2 - T_1$) (minutes)

V = Sample volume added into the reaction well (µL)

DF = Sample Dilution factor (if applicable; D = 1 for undiluted Samples)

6. Alternative calculation if the Sample Background Control (SBC) was included for the Fluorometric assay.
   a. Choose two time points (T_1 and T_2) in the linear range of the plots and obtain corresponding absorbance (RFU). for Sample (S) (i.e., RFU_{S1} and RFU_{S2}) and Sample Background Control (SBC) (i.e., RFU_{SBC1} and RFU_{SBC2}).
   b. Subtract Sample Background Control reading from Sample reading to obtain $\Delta RFU_{S\ Corrected}$:

   $\Delta RFU_{S\ Corrected}$ =

   $(RFU_{S2} - RFU_{RBC2}) - (RFU_{S1} - RFU_{RBC1})$

7. Apply the $\Delta RFU_{S\ Corrected}$ value to the Aspartate Standard Curve to determine B nmol of Aspartate generated by the Sample during the reaction time ΔT ($\Delta T = T_2 - T_1$).
8. Use Calculation shown in Step 5 to calculate the Sample Asparaginase activity corrected for sample background.

Unit Definition: One unit of Asparaginase is defined as the amount of enzyme that generates 1.0 µmol of aspartate per minute at 25 °C.

Figure 1.
Typical Aspartate Standard Curve (colorimetric detection, 0-10 nmole/well). One mole of aspartate corresponds to one mole of asparagine metabolized by asparaginase.

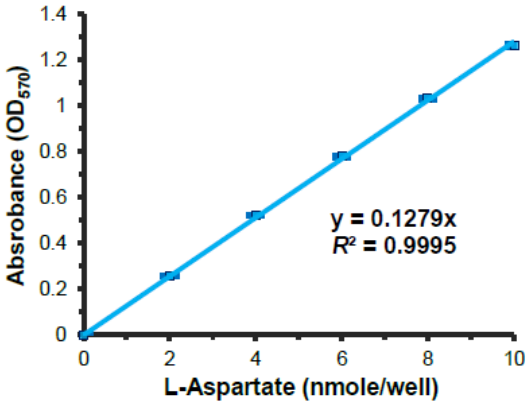


Figure 2.
Typical Aspartate Standard Curve (fluorometric detection, 0-1 nmole/well)

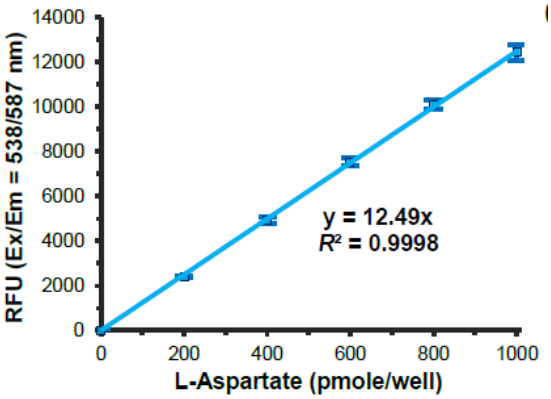
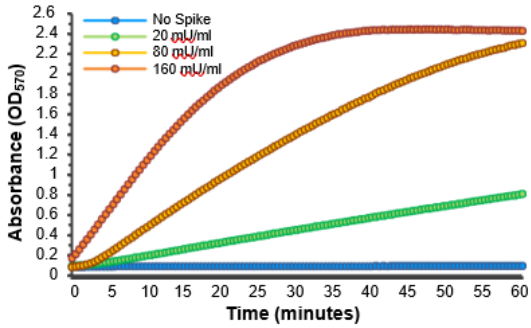


Figure 3
Reaction kinetics of asparaginase activity in human serum spiked with various amounts of asparaginase from *E. coli*. Pooled normal serum (each 5 µl per well) spiked with 0, 20, 80, or 160 mU/mL of asparaginase was assayed in colorimetric mode according to the kit protocol. Calculated sample 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%.



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