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ab273333
Asparagine Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273333
(use https://www.abcam.cn/ab273333 for china, or
https://www.abcam.co.jp/ab273333 for Japan)

For the determination of Asparagine in adherent/suspension cells and other biological fluids (plasma, serum, CSF, etc.).

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Asparagine Assay Kit (Fluorometric) (ab273333) allows for highly sensitive quantification of L-asparagine levels in biological fluids and cultured cells.

In the assay, L-asparagine is hydrolyzed to generate aspartate, which is converted to pyruvate and subsequently reacts with a fluorogenic probe to form a stable fluorophore (Ex/Em = 538/587 nm) via a series of coupled enzymatic reactions. The assay is not affected by physiological concentrations of other common amino acids and can detect asparagine levels down to 5 µM in samples.

2. Protocol Summary

Prepare all reagents and samples.

Prepare standards.

Add all samples to the appropriate wells.

Add Reaction Mix and Background Control Mix to the appropriate wells.

Incubate plate for 25 mins at RT in the dark.

Measure fluorescence in end-point mode.

Determine Asparagine using equation.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
Assay Buffer 52	25 mL	-20°C
Asparagine Enzyme Mix	1 vial	-20°C
Converter Mix L	1 vial	-20°C
Converter Mix J	1 vial	-20°C
L-Asparagine Standard	1 vial	-20°C
OxiRed™ Probe	0.2 mL	-20°C
Sample Clean-Up Mix	1 vial	-20°C

PLEASE NOTE: Assay Buffer 52 was previously labelled as Assay Buffer LII and Asparagine Assay Buffer and Converter Mix L as Conversion Enzyme Mix and Conversion Enzyme Mix Lyophilized. Converter Mix J was previously labelled as Converter Enzyme XII and Developer Enzyme Mix Lyophilized and OxiRed™ Probe as OxiRed Probe and Probe Solution. The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Multi-well fluorescence microplate reader
- Black 96-well plates with flat bottom
- 10 kDa Spin column

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

– Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 52:**
Ready to use as supplier. Allow to warm to RT before use.

9.2 **OxiRed™ Probe:**
Provided as a solution in DMSO. Divide into aliquots and store at -20°C, protected from light. Prior to use, warm solution to room temperature. After use, promptly retighten cap to minimize adsorption of airborne moisture.

9.3 **Asparagine Enzyme Mix:**
Reconstitute with 220 µl of Assay Buffer LII/Asparagine Assay Buffer. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles.

9.4 **Converter Mix L:**

Reconstitute the vial with 220 µl of Assay Buffer LII/Asparagine Assay Buffer. Divide into aliquots and store at -20°C, protected from light. Keep on ice while in use and avoid repeated freeze/thaw cycles. Upon reconstitution, use within two months.

9.5 **Converter Mix J:**
Reconstitute the vial with 220 µl of Assay Buffer 52. Divide into aliquots and store at -20°C, protected from light. Keep on ice while in use and avoid repeated freeze/thaw cycles. Upon reconstitution, use within two months.

9.6 **Sample Clean-Up Mix:**

Reconstitute the vial with 220 µl of Assay Buffer 52. Divide into aliquots and store at -20°C, protected from light. Keep on ice while in use and avoid repeated freeze/thaw cycles. Upon reconstitution, use within two months.

9.7 **L-Asparagine Standard:**

Reconstitute with 110 µl of ddH_2O for a 10 mM stock solution. Store at -20°C, stable for 4 freeze/thaw cycles.

10. Sample Preparation

10.1 Biological fluid samples (such as plasma and serum) or cell culture medium should be clarified by centrifugation at 10,000 x *g* for 5 mins at 4°C in order to separate insoluble material.

10.2 Cultured cells (~1 x 10^6) should be rapidly homogenized on ice with 100 µl ice cold Assay Buffer 52 Centrifuge at 15,000 x *g* for 10 mins at 4°C and transfer the supernatant to a new microfuge tube.

10.3 Common small molecule metabolites (such as pyruvate) and enzymes found in biological samples may interfere with the assay or increase sample background. To reduce potential interference, samples should be pretreated with Sample Cleanup Mix and deproteinized. For each test sample, add Sample Cleanup Mix to the sample at a 1:25 ratio (4 µl for every 100 µl of sample volume). Mix well and incubate samples at 37°C for 20 mins. Then transfer samples to 10 kDa Spin columns. Centrifuge treated samples at 10,000 x *g* for 10 mins at 4°C and collect the filtrate.

10.4 Once pretreated and deproteinized, samples may be stored at -80°C for future experiments for at least 2 months.

10.5 Add 2-30 µl of sample to desired well(s) in a black, flat bottom 96-well plate.

10.6 For each test sample, we recommend preparing two parallel sample wells, with one well serving as a sample background control.

10.7 Adjust the volume of all wells to 60 µl/well with Assay Buffer 52.

Δ Note: For unknown samples, we recommend performing a pilot experiment to ensure readings are within the standard curve range. Average physiological ranges for L-Asparagine are 15-60 µM in blood serum/plasma and 5-15 µM in CSF.

Δ Note: Samples with a low Asparagine concentration, we recommend running two test samples in parallel and spiking one with a known amount of L-Asparagine Standard (e.g. 400 pmol) to ensure accurate determination of L-Asparagine. Addition of a spiked sample brings the number of parallel sample wells to three.

11. Standard Curve

– Keep standards on ice while in use.

11.1 Prepare a 200 µM solution of L-Asparagine by adding 20 µl of the 10 mM L-Asparagine Standard stock to 980 µl of Assay Buffer 52.

11.2 Add 0, 2, 4, 6, 8, and 10 µl of the 200 µM working solution into a series of wells, generating 0, 400, 800, 1200, 1600 and 2000 pmol of L-Asparagine/well.

11.3 Adjust the volume to 60 µl/well with Assay Buffer 52.

Standard #	200 µM L-Asparagine Standard (µL)	Assay Buffer 52 (µL)	L-Asparagine (pmol/well)
1	0	90	0
2	2	58	400
3	4	56	800
4	6	54	1,200
5	8	52	1,600
6	10	50	2,000

12. Assay Procedure

– Keep on ice while in use.

12.1 **Reaction Mix:**
Prepare reaction mixes for Sample and Background Control wells according to the table below. Make a sufficient amount of each type of reaction mix to add 40 µl to all assay wells of that type. Remember to account for the L-Asparagine Standard curve wells and any additional wells for spiked samples (if applicable) when calculating the amount of reaction mix to prepare.

	Reaction mix (µl)	**Background Control mix (µl)**
Assay Buffer 52	33	35
Asparagine Enzyme Mix	2	---
Converter Mix L	2	2
Converter Mix J	2	2
OxiRed™ Probe	1	1

12.2 Add 40 µl of the Reaction mix to all standard curve and test sample wells**.**

12.3 Add 40 µl of Background Control mix into sample background control wells**.**

12.4 Incubate the plate for 25 mins at RT, protected from light.

12.5 Measure the fluorescence of all sample, background and standard curve wells at Ex/Em = 538/587 nm in endpoint mode.

13. Calculations

13.1 For the L-Asparagine Standard curve, subtract the zero standard (0 pmol/well) reading from all of the standard readings, plot the background-subtracted values and calculate the slope of the standard curve.

13.2 For test samples, calculate the corrected sample fluorescence (Fc) by subtracting the Sample Background RFU reading from the corresponding sample readings:

$$Fc = RFU_S - RFU_{BC}$$

13.3 For un-spiked samples, apply the Fc values to the standard curve to get B pmol of Asparagine in the well.

$$[L\ Asparagine] = \frac{B}{(V)} xD = pmol/\mu l = \mu M$$

B = Asparagine amount from the Standard Curve (pmol)
D = Dilution factor (D=1 when sample if undiluted)
V = Volume of sample added to the well (µl)

Δ Note: To quantify sample Asparagine level in spiked samples (if applicable), calculate B by subtracting the background corrected non-spiked sample reading (Fc) from the corrected spiked reading (Fc plus spike):

$$B = \left(\frac{Fc}{(Fc\ plus\ spike) - Fc}\right) X\ Asparagine\ Spike\ (pmol)$$

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

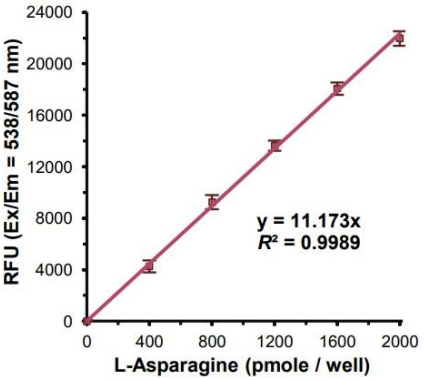

Figure 1. L-Asparagine Standard curve.

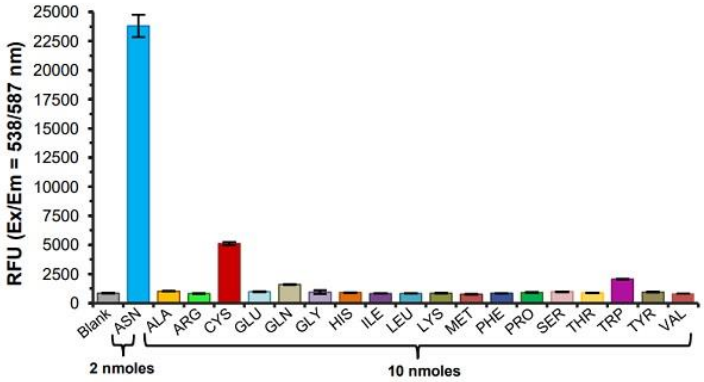

Figure 2. Specificity for detection of L-asparagine (ASN) over other common L-amino acids. At a 5- fold molar excess (10 nmole/well) versus ASN (2 nmole/well), tryptophan (TRP) gives ~5% interference and the reduced form of cysteine (CYS) gives ~20% interference (the oxidized disulfide form, which accounts for 90% of the total cysteine in biological fluids, generates ≤1% interference at 5-fold excess).

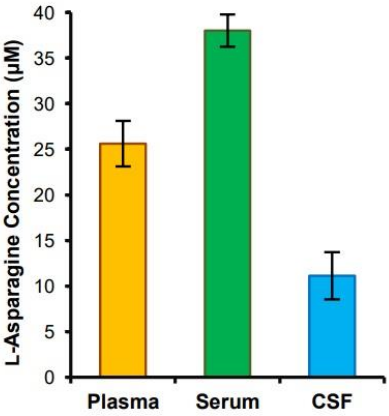

Figure 3. Estimation of total asparagine in pooled heparinized human plasma (10µl), pooled off-the-clot human serum (10µl) and pooled human CSF (15µl). Asparagine concentrations for plasma, serum and CSF samples were 25.61 ± 2.51 µM, 37.99 ± 1.77 µM and 11.11 ± 2.59 µM, respectively. Data are mean ± SEM of at least 3 replicates, samples were treated with Sample Cleanup Mix, deproteinized using 10 kDa spin columns and assayed according to the kit protocol.

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)