ab107922 - Asparaginase Activity Assay Kit (Colorimetric/Fluorometric)

Instructions for Use

For rapid, sensitive and accurate measurement of Asparaginase activity in various samples.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 7, Last Updated 5 February 2024

Table of Contents

1. BACKGROUND

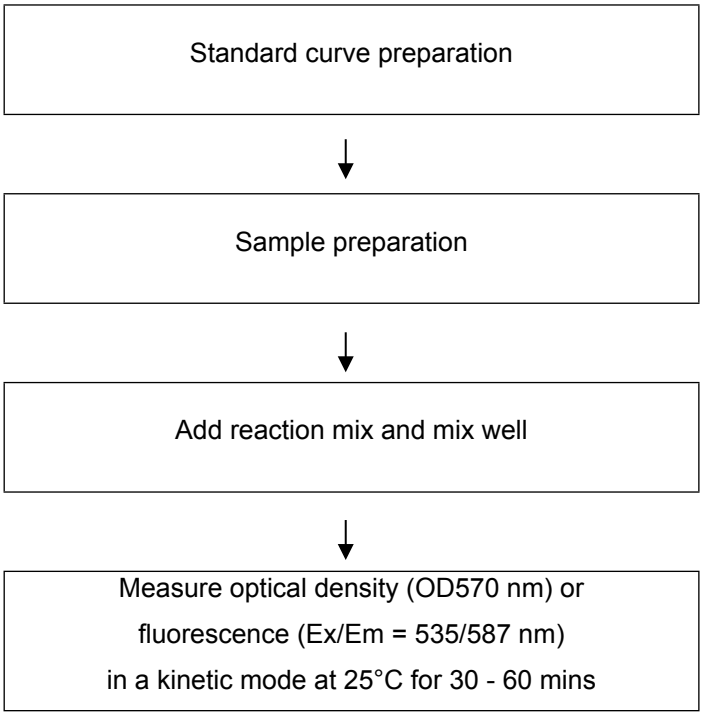
Asparaginase Activity Assay Kit (107922) provides a simple, direct and automation-ready procedure for measuring asparaginase activity in biological samples. In the assay, asparaginase hydrolyzes asparagine to generate aspartic acid, which can be detected colorimetrically (λ = 570 nm) or fluorescently (Ex/Em = 535/590 nm) using a coupled enzymatic reaction.

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).

Asparaginases can be used for different industrial and pharmaceutical purposes. The most common use is in food manufacturing as food processing aid to reduce acrylamide, a suspected carcinogen, produced in fried starchy food products. Metabolization of asparaginase prevents acrylamide formation in fried foods (Maillard reaction).

Other asparaginases are used to treat acute lymphoblastic leukemia (ALL) and some other hematopoietic neoplasms (e.g. multiple myeloma). 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.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

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. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer IV/Asparaginase Assay Buffer	25 mL	-20°C	4°C
OxiRed Probe/OxiRed Probe (in DMSO)	200 µL	-20°C	-20°C
Asparaginase Substrate Mix/Substrate Mix (lyophilized)	1 vial	-20°C	-20°C
Aspartate Enzyme Mix/Aspartate Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C
Converter Enzyme XII/Conversion Mix (lyophilized)	1 vial	-20°C	-20°C
Asparaginase Positive Control/Positive Control (lyophilized)	1 vial	-20°C	-20°C
Aspartate Standard/Aspartate Standard (100 mM)	100 µL	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader – equipped with filter for OD 570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: clear plates for colorimetric assay; black plates (clear bottoms) for fluorometric assay
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- 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.

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.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- 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.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

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

9.1 **Assay Buffer IV/Asparaginase Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

9.2 **Aspartate Standard/Aspartate Standard (100 mM):**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.3 **OxiRed Probe/OxiRed Probe – in DMSO:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light and moisture. Keep on ice while in use.

9.4 **Asparaginase Substrate Mix/Substrate Mix:**

Reconstitute in 500 µL of ddH_2O. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid multiple freeze/thaw cycles. Use within 2 months.

9.5 **Aspartate Enzyme Mix:**

Reconstitute in 220 µL Assay Buffer. Pipette up and down to completely dissolve. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid multiple freeze/thaw cycles. Use within 2 months.

9.6 **Converter Enzyme XII/Conversion Mix:**

Reconstitute in 220 µL Assay Buffer. Pipette up and down to completely dissolve. Aliquot Converter Enzyme XII/conversion mix so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid multiple freeze/thaw cycles. Use within 2 months.

9.7 **Asparaginase Positive Control/Positive Control:**

Reconstitute with 100 µL Assay Buffer. Pipette up and down to completely dissolve. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid multiple freeze/thaw cycles. Use within 2 months.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 **For the colorimetric assay:**

10.1.1 Prepare 500 µL of 1 nmol/µL (1 mM) Aspartate standard, by diluting 5 µL of Aspartate 100 mM standard in 495 µL Assay Buffer.

10.1.2 Using 1nmol/µL standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End conc Aspartate in well
1	0	150	50	0 nmol/well
2	6	144	50	2 nmol/well
3	12	138	50	4 nmol/well
4	18	132	50	6 nmol/well
5	24	126	50	8 nmol/well
6	30	120	50	10 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

10.2 **For the flurometric assay:**

10.2.1 Prepare 500 µL of 1 mM standard, by diluting 5 µL of standard in 495 µL Assay Buffer.

10.2.2 Prepare 200 µL of 0.1 nmol/µL (0.1 mM = 100 µM) Asparaginase standard by diluting 20 µL of 1 nmol/µL Standard in 180 µL Assay Buffer.

10.2.3 Using 0.1 nmol/µL standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End conc Aspartate in well
1	0	150	50	0 nmol/well
2	6	144	50	0.2 nmol/well
3	12	138	50	0.4 nmol/well
4	18	132	50	0.6 nmol/well
5	24	126	50	0.8 nmol/well
6	30	120	50	1.0 nmol/well

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 100 µL of Assay Buffer.

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Incubate sample on ice 10 – 30 minutes.

11.1.6 Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

11.1.7 Collect supernatant and transfer to a clean tube.

11.1.8 Keep on ice.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).
- Positive control well = 5 µL Asparaginase Positive Control/Positive Control (from section 9.7) (adjust volume to 50 µL/well with Assay Buffer).
- Background control sample well = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer). ***NOTE:*** *for samples containing aspartate, oxaloacetate and pyruvate as they can generate background.*

12.2 **Asparaginase Reaction Mix (COLORIMETRIC ASSAY):**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer	**40**	**44**
Asparaginase Substrate Mix/Substrate Mix	**4**	**0**
Aspartate Enzyme Mix	**2**	**2**
Converter Enzyme XII/Conversion Mix	**2**	**2**
OxiRed Probe	**2**	**2**

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1)

12.3 **Asparaginase Reaction Mix (FLUOROMETRIC ASSAY):**

Prepare 50 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer	**41.5**	**45.5**
Asparaginase Substrate Mix/Substrate Mix	**4**	**0**
Aspartate Enzyme Mix	**2**	**2**
Converter Enzyme XII/Conversion Mix	**2**	**2**
OxiRed Probe*	**0.5**	**0.5**

**For fluorometric readings, using 0.5 µL/well of the probe decreases the background readings, therefore increasing detection sensitivity.*

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1)

12.4 Add 50 µL of Reaction Mix to each well (standard, positive control, background control and samples).

12.5 Mix well.

12.6 Measure output on a microplate reader in a kinetic mode after 10 minutes, every 2 – 3 minutes, for 30 - 60 minutes at 25°C protected from light.

- Colorimetric assay: measure OD570 nm.
- Fluorometric assay: measure Ex/Em = 535/587nm.

There is an initial lag phase of 10 minutes that can lead to underestimation of the Asparaginase activity. We recommend measuring activity after the initial 10 min incubation.

NOTE: *Sample incubation time can vary depending on asparaginase activity in the samples. We recommend measuring absorbance/fluorescence in kinetic mode and then choosing two time points (T_1 and T_2) after the initial lag phase, during the linear range.*

RFU/OD value at T_2 should not exceed the highest OD in the standard curve. For standard curve, do not subtract A_1 from A_2 reading.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of Aspartate.

13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Activity of Asparaginase is calculated as:

$$\mathbf{\Delta A_{570nm} / \Delta RFU_{535/575nm} = (A_2 - A_{2BG}) - (A_1 - A_{1BG})}$$

Where:

A1 is the sample reading at time T1.

A1BG is the background control sample at time T1.

A2 is the sample reading at time T2.

A2BG is the background control sample at time T2.

13.6 Use the $\Delta A_{570nm} / \Delta RFU_{535/575nm}$ to obtain B nmol of aspartate generated by asparaginase during the reaction time ($\Delta T = T_2 - T_1$).

13.7 Concentration of aspartate in the test samples is calculated as:

$$Asparaginase\ Activity = \left(\frac{B}{\Delta T x\, V}\right) * D$$

Asparaginase activity= nmol/min/mL =mU/mL

Where:

B = Amount of aspartate from aspartate Standard Curve.

ΔT = reaction time (min).

V = original sample volume added into the reaction well (in mL).

D = sample dilution factor.

Unit Definition:

1 Unit Asparaginase = amount of asparaginase which generates 1.0 µmol of aspartate per min at 25°C.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

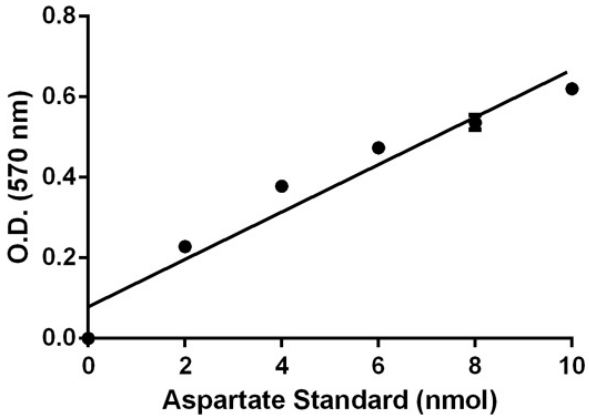


Figure 1. Typical asparaginase standard calibration curve using colorimetric reading.

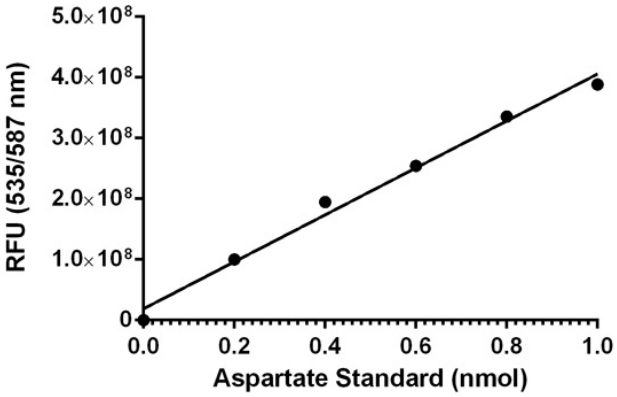


Figure 2. Typical asparaginase standard calibration curve using fluorometric reading.

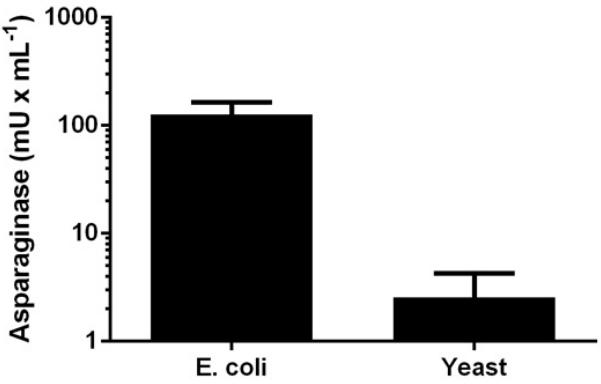


Figure 3. Asparaginase measured in cell lysates showing quantity (mU) per mL of tested sample.

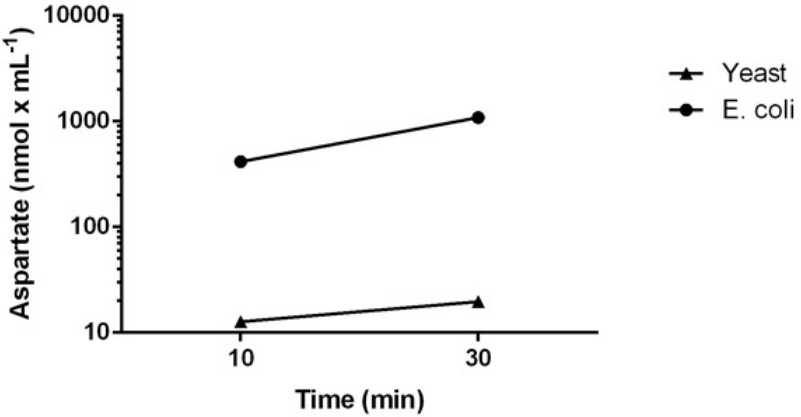


Figure 4. Aspartate measured in cell lysates after 10 min and 30 min incubation time showing quantity (nmol) per mL of tested sample.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, probe, Asparaginase Substrate Mix/substrate mix, Converter Enzyme XII/conversion mix and enzyme mix (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard, positive control (50 µL) and samples (50 µL).
- Prepare Asparaginase Reaction Mix (Number samples + standards + 1).

Component	Colorimetric / Bkg Reaction Mix (µL)	Fluorometric / Bkg Reaction Mix (µL)
Assay Buffer	**40 / 42**	**41.8 / 43.8**
Asparaginase Substrate Mix/Substrate Mix	**4 / 4**	**4 / 4**
Aspartate Enzyme Mix	**2 / 2**	**2 / 2**
Converter Enzyme XII/Conversion Mix	**2 / 0**	**2 / 0**
OxiRed Probe	**2 / 2**	**0.2 / 0.2**

- Add 50 µL to Reaction Mix to standard, control and sample wells and mix well.
- After initial lag phase (10 minutes), incubate plate at 25°C during 30 minutes and read absorbance at OD=570 nm or fluorescence at Ex/Em= 535/587 nm in a kinetic mode.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17.FAQ

18. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure

- RIPA - contains SDS which can destroy/decrease the activity of the enzyme.
- Presence of aspartate, oxaloacetate or pyruvate can generate high background – please perform the relevant control.

19. 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)