ab107922 – Asparaginase Activity Assay Kit (Colorimetric/Fluorometric)

For rapid, sensitive and accurate measurement of Asparaginase activity in various samples.
For research use only - not intended for diagnostic use.

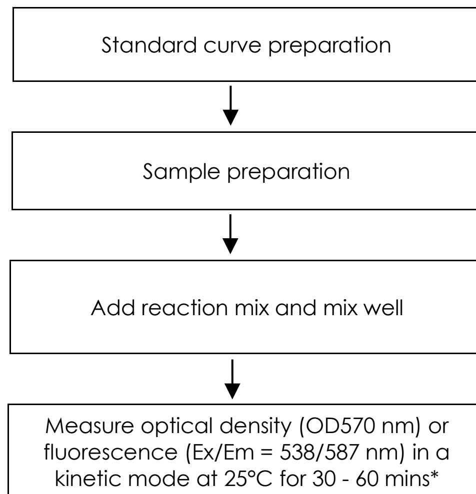
For overview, typical data and additional information please visit:
http://www.abcam.com/ab107922 (use http://www.abcam.cn/ab107922 for China, or http://www.abcam.co.jp/ab107922 for Japan)

Background:

Asparaginase Activity Assay Kit (107922) provides a simple, direct and automation-ready procedure for measuring asparaginase activity in biological samples including cell lysates. In the assay, asparaginase hydrolyzes asparagine to generate aspartic acid, which can be detected colorimetrically (λ = 570 nm) or fluorescently (Ex/Em = 538/587 nm) using a coupled enzymatic reaction.

Assay Summary:

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



*Incubation time given in this summary is for guidance only.

QUICK ASSAY PROCEDURE

- Prepare standard, probe, Asparaginase Substrate Mix, Converter Mix J and Aspartate Enzyme Mix (aliquot if necessary); get equipment ready.
- Prepare samples in duplicate
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Set up plate for standard (50 µL) and samples (50 µL).
- Prepare Asparaginase Reaction Mix (Number samples + standards + 1).
- Add 50 µL Asparaginase Reaction Mix to each well.
- Measure plate at OD 570 nm for colorimetric assay or Ex/Em= 538/587 for fluorometric assay in kinetic mode at RT for 30-60 min.

Precautions & Limitations:

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.
- 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.

Storage and Stability:

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Assay Buffer 4	25 mL	-20°C	4°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
Asparaginase Substrate Mix	1 vial	-20°C	-20°C
Aspartate Enzyme Mix	1 vial	-20°C	-20°C
Converter Mix J	1 vial	-20°C	-20°C
Asparaginase Positive Control	1 vial	-20°C	-20°C
Aspartate Standard	0.1 mL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 4 was previously labeled as Assay Buffer IV and Asparaginase Assay Buffer, and Converter Mix J as Converter Enzyme XII and Conversion Mix (lyophilized). OxiRed™ Probe was previously labeled as OxiRed Probe (in DMSO), and Asparaginase Substrate Mix as Substrate Mix (lyophilized), and Asparaginase Positive Control as Positive Control (lyophilized). The compositions have not changed.

Materials Required, Not Supplied:

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

- Microplate reader capable of measuring absorbance (OD) at 570 nm (colorimetric) or fluorescence at Ex/Em = 538/587 nm (fluorometric)
- 96 well clear plate with clear flat bottom (colorimetric assay) / 96 well black plate with flat bottom (fluorometric assay)
- Orbital shaker
- Microcentrifuge
- Dounce homogenizer (if using tissues or cells)
- 1 x PBS pH 7.4
- Heat block or water bath
- MilliQ water or other type of double distilled/deionized water (ddH_2O)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

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

Aspartate Standard: 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.

OxiRed™ Probe: Ready to use as supplied. Keep on ice during the assay. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Keep at room temperature during the assay. Store at -20°C and protect from light and moisture. Once the probe is opened and thawed, it is stable for at least 3 additional freeze/thaw cycles but should be used within two months. After use, promptly retighten the cap to minimize adsorption of airborne moisture.

Asparaginase 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.

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.

Converter Mix J: Reconstitute in 220 µL Assay Buffer. Pipette up and down to completely dissolve. Aliquot Converter Mix J 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.

Asparaginase 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.

Sample Preparation:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

1. 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 at -80°C. Alternatively, snap freeze your samples in liquid nitrogen upon extraction and store them 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.
2. Interferences:
   - RIPA Buffer - 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.

Cells (adherent or suspension) samples:

1. Harvest the number of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).
2. Wash cells in cold PBS.
3. Resuspend cells in 100 µL of Assay Buffer.
4. Homogenize cells using a Dounce homogenizer sitting on ice.
5. Centrifuge sample for 10 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- Each dilution has enough standard to set up duplicate readings (2 x 50 µL).
- 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.

Prepare serial dilution of Aspartate Standard as follows:

1. 1 mM dilution: Add 5 µL of Aspartate 100 mM standard to 495 µL Assay Buffer. Gently pipette up and down a few times to ensure all standard is removed from tip. Mix well by inversion. **Use this to prepare standard curve for colorimetric assay.**
2. 0.1 mM dilution: Transfer 20 µL of 1 mM dilution to 180 µL Assay Buffer. Gently pipette up and down and then mix well by inversion. **Use this to prepare standard curve for fluorometric assay.**

For colorimetric assay: Using 1 mM Aspartate standard, add 0, 2, 4, 6, 8, 10 µl into a series of standards wells. Adjust volume to 50 µl/well with Assay Buffe IV to generate 0, 2, 4, 6, 8, and 10 nmol/well of the Aspartate Standard, or prepare standard curve dilution as described in the table below in a microplate or microcentrifuge tubes (sufficient for duplicate standard curves):

For fluorometric assay: Using 0.1 mM Aspartate Standard, add 0, 2, 4, 6, 8, 10 µl into a series of standards wells. Adjust volume to 50 µl/well with Assay Buffer 4 to generate 0, 0.2, 0.4, 0.6, 0.8, and 1 nmol/well of the Aspartate Standard, or prepare standard curve dilution as described in the table below in a microplate or microcentrifuge tubes(sufficient for duplicate standard curves):

Standard #	Volume of 1 mM or 0.1 mM Standard (µL)*	Assay Buffer 4 (µL)	Final volume standard in well (µL)	End Aspartate Amount (nmoles/well) Colorimetric Assay	End Aspartate Amount (nmoles/well) Fluorometric Assay
1	0	125	50	0	0
2	5	120	50	2	0.2
3	10	115	50	4	0.4
4	15	110	50	6	0.6
5	20	105	50	8	0.8
6	25	100	50	10	1

***NOTE: For colorimetric assay use 1 mM standard, for fluorometric assay use 0.1 mM standard**

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.

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 4).
   - Positive control well = 5 µL Asparaginase Positive Control (adjust volume to 50 µL/well with Assay Buffer 4).
   - Background control sample well = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).
     *Note: Background controls are required for samples containing aspartate, oxaloacetate and pyruvate as they can increase background.
2. Each well (standards, samples, and controls) requires 50 µL of Reaction Mix as shown in the table below. To ensure consistency, use the table below to prepare a Master Mix of the appropriate Reaction Mix for your assay using the following calculation:
   X µL component x (Number reactions +1).

Component	Colorimetric Assay Reaction Mix (µL)	Colorimetric Background Reaction Mix (µL)	Fluorometric Assay Reaction Mix (µL)	Fluorometric Background Reaction Mix (µL)
Assay Buffer 4	40	44	41.5	45.5
Asparaginase Substrate Mix	4	0	4	0
Aspartate Enzyme Mix	2	2	2	2
Converter Mix J	2	2	2	2
OxiRed™ Probe	2	2	0.5	0.5

*NOTE: For fluorometric assays, using 0.5 µL/well of the OxiRed™ Probe will reduce background and improve assay sensitivity.

3. Mix Master Reaction Mixes by inversion. Add 50 µL of the Master Reaction Mix to each sample and standard well. Add 50 µL of the Master Background Reaction Mix to each sample background well Use a clean tip for each well. (final well volume 100 µL)
4. Mix and immediately begin taking kinetic measurements for 30 - 60 minutes at 25°C. Measure absorbance on a microplate reader at OD 570 nm for Colorimetric assays or fluorescence at Ex/Em= 538/587 nm for Fluorometric assays.
   *Note: There may be an initial lag phase of up to 10 minutes that can lead to underestimation of the Asparaginase activity if time points are not chosen appropriately.
   *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.

Calculations:

- 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.

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all readings. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the final concentration of aspartate (nmol).
4. Calculate the equation of the standard curve using a linear regression and determine the slope.
5. Determine the ΔAbsorbance for colorimetric assays (or similarly ΔRFU for fluorometric assays) for each sample using the formula below.

$$\Delta A_{570nm} = (A_2 - A_{2BG}) - (A_1 - A_{1BG})$$

Where:
A_1 = sample reading at time T_1.
A_{1BG}= background control sample at time T_1.
A_2 = sample reading at time T_2.
A_{2BG}= background control sample at time T_2.

6. Apply the corrected ΔA_{570nm} or $\Delta RFU_{538/587nm}$ to the standard curve to obtain B nmol of aspartate generated by asparaginase during the reaction time

$$B = \left(\frac{\Delta A_{570\,nm} \text{ or } \Delta RFU_(538/587nm) - (y - intercept)}{Slope}\right)$$

7. Concentration of aspartate in the test samples is calculated as:

$$Asparaginase\ Activity = \left(\frac{B}{\Delta T \times V}\right) \times D = \text{nmol/min/mL} = \text{mU/mL}$$

Where:
B = amount of aspartate from aspartate Standard Curve (in nmoles).
ΔT = reaction time of linear phase (in minutes). ($\Delta T = T_2 - T_1$).
V = original sample volume added into the reaction well (in mL).
D = sample dilution factor (prior to reaction well set up).

Unit Definition:
1 Unit Asparaginase = amount of asparaginase which generates 1.0 µmole of aspartate per min at 25°C under the assay conditions.

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

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