ab325359 – Argininosuccinate Synthetase (ASS1) Inhibitor Screening Kit (Fluorometric)

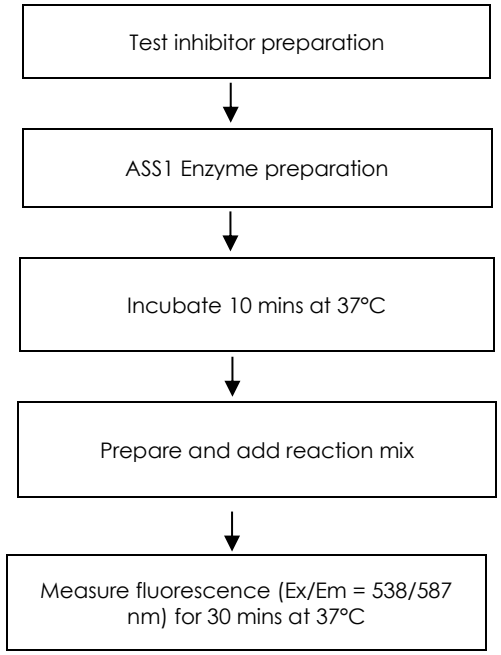
For rapid, sensitive and reliable test suitable for high-throughput screening of ASS1 Inhibitors.
For research use only - not intended for diagnostic use.

Background:

ASS1 Inhibitor Screening Kit (Fluorometric) (ab325359) provides a simple, rapid method for screening inhibitors of the enzyme argininosuccinate synthase 1 (ASS1). ASS1 is a rate-limiting urea cycle enzyme responsible for biosynthesis of arginine from citrulline and aspartate. As arginine depletion is a promising therapeutic strategy for cancer management, targeted inhibition of ASS1 may decrease tumor proliferation and metastasis in certain malignancies.

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.



QUICK ASSAY PROCEDURE

- Reconstitute ASS1 Enzyme, Buffer Supplement I, Developer Mix A, ASS1 Substrate, and ASS1 Inhibitor. Thaw OxiRed™ Probe, Assay Buffer 45, Enzyme Mix X, and Citrulline Cofactor Mix. (aliquot if necessary); get equipment ready
- Prepare test inhibitor samples in duplicate.
- Prepare ASS1 Enzyme and ASS1 Inhibitor.
- Set up plate for test inhibitors, ASS1 Inhibitor, and ASS1 Enzyme (50 µL). Incubate plate for 10 minutes at 37°C
- Prepare Reaction Mix and add 50 µL Reaction Mix to each well.
- Measure plate Ex/Em= 538/587 nm for 30 minutes at 37°C.

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. 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)	Long term storage temperature
Assay Buffer 45	25 mL	-20°C	+4°C to -20°C
OxiRed™ Probe (in DMSO, anhydrous)	200 µL	-20°C	-20°C
ASS1 Enzyme	1 vial	-20°C	-20°C
Enzyme Mix X	200 µL	-20°C	-20°C
Buffer Supplement I	1 vial	-20°C	-20°C
Citrulline Cofactor Mix	200 µL	-20°C	-20°C
Developer Mix A	1 vial	-20°C	-20°C
ASS1 Substrate	1 vial	-20°C	-20°C
ASS1 Inhibitor	1 vial	-20°C	-20°C

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 fluorescence at Ex/Em = 538/587 nm
- 96 well black plate with flat bottom
- Microcentrifuge
- MilliQ water or other type of double distilled/deionized water (ddH2O)

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 45, **Enzyme Mix X**, and **Citrulline Cofactor Mix** are ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C and protect from light and moisture.

OxiRed™ Probe: Ready to use as supplied. Warm by placing in a 37°C bath for 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.

ASS1 Enzyme: Reconstitute with 50 µL of Assay Buffer 45. Keep on ice during the assay. Use within two months.

Buffer Supplement I: Reconstitute with 220 µL of ddH2O. Keep on ice during the assay. Use within two months

Developer Mix A: Reconstitute each with 220 µL of Assay Buffer 45. Keep on ice during the assay. Use within two months

ASS1 Substrate: Reconstitute with 100 µL of ddH2O to generate a 100 mM working solution. Keep on ice during the assay.

ASS1 Inhibitor: Reconstitute with 220 µL of Assay Buffer 45 to generate a 100 mM working solution. Keep on ice during the assay. Use within two months

Test Inhibitor Preparation:

- Dissolve candidate test compounds in a suitable solvent.
- Dilute to 10X concentration with Assay Buffer 45.
- Add 10 µL of diluted test compound(s) to each Test Inhibitor well.

*NOTE: Preferred final solvent concentration should not be more than 2% by volume. If solvent exceeds 2%, include a Solvent Control to test the effect of the solvent on enzyme activity.

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 controls and samples in duplicate.

1. **Set up reaction wells:**
   - Add 2 µL of the ASS1 Enzyme stock solution to all test compounds/inhibitors, inhibitor control, and enzyme control wells.
   - Add 10 µL of 100 mM ASS1 Inhibitor to all inhibitor control wells.
   - Adjust total well volume of all wells to 50 µL with Assay Buffer 45.
   - Add 50 µL of Assay Buffer 45 to black wells for Background Control.

	Test Inhibitor (µL)	100mM ASS1 Inhibitor (µL)	ASS1 Enzyme (µL)	Assay Buffer 45 (µL)
Test Inhibitor Wells	10	0	2	38
Inhibitor Control Wells	0	10	2	38
Enzyme Control Wells	0	0	2	48
Background Control Wells	0	0	0	50

Mix well and incubate plate with plate cover at 37°C for 10 minutes.

*NOTE: The volume of all wells including Test Compound(s), ASS1 Inhibitor, ASS1 Enzyme, and Background Control is 50 µL.

2. **Prepare Reaction Mix**:
   - Prepare 50 µL of Reaction Mix for each well (including Background Control) following the table below:

Kit Component	Volume (µL) per reaction well
Assay Buffer 45	39
Enzyme Mix X	2
Buffer Supplement I	2
Developer Mix A	2
Citrulline Cofactor Mix	2
ASS1 Substrate	2
OxiRed™ Probe	1

Mix enough reagents for the number of wells in use. To prepare a bulk of the Reaction Mix, we recommend the following calculation:

X µL kit component * (number of wells + 1)

- Add 50 µL of Reaction Mix to all wells.
- Immediately measure fluorescence Ex/Em = 538/587 nm on a microplate reader in kinetic mode for 30 minutes at 37°C.

Calculations:

1. Average the duplicate reading for each test compound.
2. Choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding fluorescence values (RFU_1 and RFU_2).
3. Calculate the slope for all samples (S), including Enzyme Control (EC), by dividing the net ΔRFU (RFU_1 - RFU_2) values by the time ΔT ($T_1 - T_2$).
4. Calculate the % Relative Inhibition as follows:

$$\%\ Relative\ Inhibition = \left(\frac{Slope\ EC - Slope\ S}{Slope\ EC}\right) X\ 100$$

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

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)