ab283411– ASPG Inhibitor Screening Kit (Fluorometric)

For the screening of potential ASPG inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283411

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
ASPG Assay Buffer	50 mL	-20°C
ASPG Enzyme	100 µL	-20°C
ASPG Substrate (in EtOH)	100 µL	-20°C
DTNB (in DMSO)	100 µL	-20°C
Inhibitor	40 µL	-20°C

Materials Required, Not Supplied

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

- Multi-well fluorescence microplate reader
- 96-well clear microtiter plates with flat bottom

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

ASPG Assay Buffer: Warm to room temperature (RT) before use.

ASPG Enzyme: Aliquot and store at -20°C. Avoid repeated freeze-thaw. Use within six months.

ASPG Substrate (in EtOH): Divide into aliquots and store at -70°C. Avoid repeated freeze and thaw cycles. Keep on ice while in use. Use within two months.

DTNB (in DMSO): Before use, thaw at RT. Store at -20°C.

Inhibitor (in DMSO): Warm to room temperature (RT) before use.

Assay Protocol

Enzyme Solution Preparation:

1. Before the assay, dilute ASPG Enzyme 1:5 with Assay Buffer. i.e. take 40 µl ASPG Enzyme to 160 µl Assay Buffer, Mix well. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl ASPG Enzyme Solution:

ASPG Assay Buffer	45 µL
Diluted ASPG Enzyme	5 µL

2. Mix well and add 50 µL/well of ASPG Enzyme Solution to desired wells.

Δ Note: *Do not save the diluted ASPG Enzyme. Discard it after use.*

Screen Compounds, Inhibitor Control, and Blank Control Preparation:

1. Dissolve candidate inhibitors into appropriate solvent at 100X the highest final concentration to be tested.
2. Dilute to 4X the desired test concentration with ASPG Assay Buffer.
3. Add 50 µl diluted test inhibitor, or ASPG Assay Buffer into wells containing ASPG Enzyme, as Sample Screen [S], or Enzyme Control [EC] (no inhibitor).
4. Dilute Inhibitor (Methyl Arachidonyl Fluorophosphonate) by adding 5 µl of ASPG Inhibitor Control to 245 µl of ASPG Assay Buffer.
5. Add 50 µl of diluted Inhibitor Control into desired well(s).
6. Adjust the volume of Sample, Enzyme Control, and Inhibitor Control wells to 100 µl/well with ASPG Assay Buffer.
7. Incubate at room temperature for 5 minutes.

Δ Note: *Use diluted ASPG Inhibitor Control within 4 hours.*

ASPG Substrate Solution Preparation:

1. For each well prepare 50 µL Substrate Solution containing:

	Substrate Solution
ASPG Substrate	2 µL
DNTB Solution	1 µL
ASPG Assay Buffer	47 µL

2. Mix and add 50 µl of Substrate solution into each well. Mix well.
3. Incubate for more than 60 minutes at 37°C, protected from light.

Measurement

Measure absorbance at OD 412 nm.

Calculation:

1. Set the absorbance of enzyme control (EC) as 100%, and calculate the relative % inhibition of test inhibitors (S) as follows:

$$\% \text{ Inhibition} = \frac{OD\ 412\ nm\ of\ EC - OD\ 142\ nm\ of\ S}{OD\ 412\ nm\ of\ EC} \times 100$$

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)

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