ab308245 – Urease Inhibitor Screening Kit (Colorimetric)

Screening or characterizing urease inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

Store kit at -20 °C, protected from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

Materials Supplied

Item	Quantity	Storage Condition after preparation
Urease Assay Buffer	25 ml	4 °C or -20 °C
Ammonia Reagent 1 (Avoid light)	8 ml	4 °C
Ammonia Reagent 2	4 ml	4 °C
Urea	250 µl	-20°C
Urease Enzyme	1 vial	-20°C
Urease Inhibitor (Avoid light)	50 µl	-20°C

Materials Required, Not Supplied

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

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer
- DMSO

Reagent Preparation

Urease Assay Buffer: Store at 4 °C or -20 °C. Bring to room temperature (RT) before use.

Ammonia Reagent 1 & Ammonia Reagent 2: Store at 4 °C. Bring to RT before use.

Urea (1.5 M): Store at -20 °C. Bring to RT before use.

Urease Enzyme: Reconstitute the vial in 220 µl Urease Assay Buffer. Divide into aliquots and store at -20 °C. Stable for two months. Keep on ice during use.

Urease Inhibitor (5 mM in DMSO): Warm to RT. Divide into aliquots and store at -20 °C.

Urease Inhibitor Screening Protocol

Urease Enzyme Dilution:

Prepare 1:500 dilution of the Urease Enzyme using Urease Assay Buffer. Mix thoroughly and keep on ice. Add 20 µl of diluted Urease Enzyme into the desired wells of a 96-well clear plate labelled as Sample, Solvent Control, Inhibitor Control and Enzyme Control. Adjust the volume of all wells to 25 µl using Urease Assay Buffer.

Test Inhibitor(s):

Dissolve Test Inhibitor(s) in an appropriate solvent to make 100X stock solution. Dilute the stock Test Inhibitor to 4X using Urease Assay Buffer. Add 25 µl of diluted Test Inhibitor into the Sample well(s). Add 25 µl of 4X Solvent (4X final well solvent concentration) into the Solvent Control well.

Δ Note: *Solvents used to solubilize the Test Inhibitor(s) might affect the enzymatic activity. Thus, prepare a Solvent Control well with the same final concentration of solvent used to dissolve the Test Inhibitor(s).*

Enzyme Control, Background Control and Inhibitor Control Preparation

Add 25 µl of Urease Assay Buffer to the Enzyme Control well. For Background Control, add 50 µl of Urease Assay Buffer in a separate well. To the Inhibitor Control well, add 2 µl of 5 mM Urease Inhibitor and adjust the volume to 50 µl/well by adding 23 µl Urease Assay Buffer. At this stage, the volume of all wells including Sample, Solvent Control, Inhibitor Control, Enzyme Control and Background Control is 50 µl/well.

IC_{50} estimation (Optional):

Prepare several dilutions of the Test Inhibitor(s) in Urease Assay Buffer while maintaining constant final Solvent Concentration in all wells. Add 25 µl of each dilution into the designated wells.

Urease Substrate Mix Preparation

Mix enough Urease Substrate Mix for the number of assays to be performed. Prepare 50 µl Urease Substrate Mix per reaction as shown below.

	Urease Substrate Mix
Urease Assay Buffer	47.5 µl
Urea	2.5 µl

Add 50 µl Urease Substrate Mix to Sample(s), Solvent Control, Inhibitor Control, Enzyme Control and Background Control wells, mix well. The total reaction volume is 100 µl/well. Incubate plate at 37 °C for 30 min.

Measurement

Add 80 µl of Ammonia Reagent 1 to each well and mix. Add 40 µl of Ammonia Reagent 2 to each well and mix again. Incubate at 37 °C for 30 min, protected from light. Measure the OD at 670 nm in a microplate reader in endpoint mode.

Calculation

Obtain the corrected absorbance (ΔOD) for all Test Samples [S], Enzyme Control [EC], Solvent Control [SC] and Inhibitor Control [IC] wells by subtracting the reading of the [BC] wells from all readings. If the [SC] reading is significantly different from [EC], then use the corrected [SC] instead of the [EC] in the formula below. Calculate the % Relative Inhibition as shown below.

$$\% \text{ Relative Inhibition} = \frac{\Delta OD\ of[\ EC] - \Delta OD\ of\ [S]}{\Delta OD\ of[EC]} \text{ X } 100$$

Technical Support

For all technical or commercial enquiries please go to:

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