Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Urease Inhibitor Screening Kit (Colorimetric)

03/21

(Catalog # K2079-100; 100 assays; Store at -20 °C)

I. Introduction:

Urease (Urea Aminohydrolase, E.C.3.5.1.5) is a nickel-dependent metalloenzyme that catalyzes the hydrolysis of urea to ammonia and carbon dioxide. Urease is produced by plants, bacteria, fungi and some invertebrates. Bacterial urease is of special importance since it is a major virulence factor caused by bacteria during infections. It is essential in the colonization of host organism and in the maintenance of bacterial cells in tissues. Ureases hydrolyze urea secreted in the blood and saliva to release ammonia, which is the major factor contributing to infections such as urinary tract and gastroduodenal infections, urinary catheter encrustation, stomach cancer, kidney stone formation, pneumonia etc. Thus, controlling the ureolytic activity of microorganism is a promising target to treat diseases caused by bacterial ureases. **BioVision's Urease Inhibitor Screening Kit** provides a quick, easy and sensitive assay to screen and characterize potential urease inhibitors. In this assay, urease of *Canavalia ensiformis* is used to screen urease inhibitors. The production of ammonia is measured at 670 nm using a modified Berthelot method and is applied to determine the inhibition efficiency of the screened compounds. A control Urease Inhibitor is also included in the kit.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} \text{Ammonia} + CO_2$$

II. Application:

- Screening or characterizing urease inhibitors.

III. Kit Contents:

Components	K2079-100	Cap Code	Part Number
Urease Assay Buffer	25 ml	WM	K2079-100-1
Ammonia Reagent 1	8 ml	Amber	K2079-100-2
Ammonia Reagent 2	4 ml	Clear	K2079-100-3
Urea	250 µl	Red	K2079-100-4
Urease Enzyme	1 vial	Blue	K2079-100-5
Urease Inhibitor	50 µl	Yellow	K2079-100-6

IV. User Supplied Reagents and Equipment:

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

V. Storage Conditions and Reagent Preparation:

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

- **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.

VI. Urease Inhibitor Screening Protocol:

1. 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 labeled as **Sample**, **Solvent Control**, **Inhibitor Control** and **Enzyme Control**. Adjust the volume of all wells to 25 µl using Urease Assay Buffer.

2. 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. **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).

3. 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.

4. 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.

5. 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.

6. 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.

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

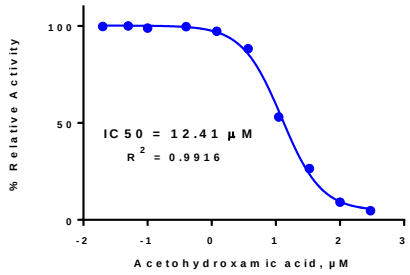

Figure: Inhibition of Urease activity by Acetohydroxamic acid. IC_{50} was calculated to be 12.41 ± 4 µM. Assay was performed in duplicates following the kit protocol.

VII. Related Products:

Urease Activity Assay Kit (Colorimetric) (Cat. # K378-100)
Urea Colorimetric Assay Kit II (Cat. # K376-100)
Uric Acid Colorimetric/Fluorometric Assay Kit II (Cat. # K608-100)
Uricase Activity Assay Kit (Fluorometric) (Cat. # K734-100)
Ammonia Colorimetric Assay Kit (Cat. # K370-100)
Ammonia Colorimetric Assay Kit II (Cat. # K470-100)
Phenylalanine Ammonia-Lyase Activity Assay Kit (Fluorometric) (Cat. # K2055-100)
Homocysteine Lyase Activity Assay Kit (Fluorometric) (Cat. # K2026-100)
Furin Activity Assay Kit (Fluorometric) (Cat. # K2076-100)
Furin Inhibitor Screening Kit (Fluorometric) (Cat. # K2069-100)

FOR RESEARCH USE ONLY! Not to be used on humans.