ab284529 – Homocysteine Lyase Activity Assay Kit (Fluorometric)

For the quantitative measurement of HCYase activity in bacterial samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284529

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 1 year.

Materials Supplied

Item	Quantity	Storage Condition
HCY Assay Buffer	25 mL	-20°C
AMC Standard	100 µL	-20°C
HCY Probe	500 µL	-20°C
HCY Substrate	5 vials	-20°C
Positive Control	1 vial	-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 white flat-bottom plate
- Multi-well spectrophotometer
- Bacterial lysis buffer

Reagent Preparation

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

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

HCY Substrate: Reconstitute each vial in 1.1 ml dH_2O. Mix well and keep at RT. Discard the reconstituted substrate solution after 2-3 days. Store the non-reconstituted vials at -20°C.

HCY Probe: Ready to use. Provided as a solution in DMSO. Divide into aliquots and store at -20°C, protected from light. Warm solution to RT before performing the assay.

AMC Standard: Ready to use. Warm to RT before use.

Positive Control: Reconstitute in 100 µl dH_2O. Mix well. Keep on ice. Divide into aliquots and store at -20°C. Avoid repeated freeze-thaw cycles.

Assay Protocol

Sample Preparation:

1. Grow bacteria according to the experimental conditions. Harvest the bacterial cells by centrifugation.
2. Transfer ~100 mg of cell pellet into an Eppendorf tube and add 1 ml of bacterial lysis buffer to resuspend the cells. Sonicate for 1-2 min on ice and centrifuge at 10,000 x g for 15 min at 4°C. Collect the clear cell supernatant.
3. Dilute the lysate to 1:50 dilution using HCY Assay Buffer.
4. Add 2-5 µl of the diluted Sample into desired wells of a white, flat bottom 96-well plate. Adjust the volume of each well to 100 µl using HCY Assay Buffer.
5. For **Positive Control [PC]** well, add 5 µl of the reconstituted Positive Control into the desired well(s). Adjust the volume to 100 µl/well with HCY Assay Buffer.
6. For **Background Control [BC]** well, add 100 µl of HCY Assay Buffer.

Δ Notes:

a. *Samples should be clarified by centrifugation prior to use to eliminate any cell debris.*
b. *We recommend using the Samples immediately for activity analysis. Otherwise, store the Sample(s) at -80°C for future experiments.*
c. *For Unknown Samples, we suggest testing several dilutions to ensure that the readings are within the Standard Curve range (0.5-7 µM or 10-1400 pmol/well). Samples with higher levels of HCYase may be diluted with HCY Assay Buffer.*

Standard Curve Preparation:

1. Prepare 10 fold dilution of the AMC Standard by adding 10 µl of the AMC Standard with 90 µl of HCY Assay Buffer.
2. Add 0, 2, 4, 6, 8, 10, 12 and 14 µl of diluted AMC Standard into a series of wells of a 96-well white plate to generate 0, 200, 400, 600, 800 and 1000, 1200 and 1400 pmol/well AMC Standard.
3. Adjust the volume of each well to 200 µl with HCY Assay Buffer. Mix well and measure the fluorescence at Ex/Em = 368/460 nm in kinetic mode for 10 min.

Reaction Mix:

1. Add 50 µl of HCY Substrate to all the wells including Sample(s), PC and BC wells. Incubate the plate at RT for 30 min. Meanwhile, prepare the Reaction Mix. Mix enough reagents for the number of assays to be performed. For each Sample, PC and BC wells, prepare 50 µl Reaction Mix containing:

Item	Reaction Mix
HCY Assay Buffer	46 µL
HCY Probe	4 µL

2. Mix well. Add 50 µl Reaction Mix to all the wells including Sample(s), PC and BC. The final volume in these wells should be 200 µl/well.

Measurement

Measure the fluorescence of Sample(s), PC and BC all wells at Ex/Em = 368/460 nm in kinetic mode at 1 min interval for 30-60 min at RT.

Calculation:

1. Subtract the 0 Standard RFU readings from all Standard RFU readings. Plot the AMC Standard Curve.
2. For each Sample type, choose any two time points within the linear range of the curve (t_1 and t_2).
3. For Sample wells, calculate the net fluorescence signal (F) by subtracting the Background RFU reading (RFU_{BC}) from the Sample RFU readings (RFU_{Sample}) for the chosen t_1 and t_2 time points: [$F = RFU_{Sample} - RFU_{BC}$].
4. Apply the net fluorescence signal to the AMC Standard Curve to get B pmol of AMC generated during the reaction time ($\Delta t = t_2 - t_1$).
5. Calculate the total amount of HCYase in the Sample using the following equation:

$$\text{Homocysteine Lyase Activity} = \frac{B}{V \; X \; \Delta T} \times D = mU/mL$$

Where: **B** = Amount of AMC calculated from the AMC Standard Curve (pmol)
V = Volume of sample added to the well (ml)
ΔT = Time between t_2 and t_1 in minutes
D = Sample dilution factor (D=1 for undiluted samples)

Unit Definition: One Unit of HCYase Activity is the amount of enzyme that generates 1 µmole of H_2S per minute at RT.

Technical Support

For all technical or commercial enquiries please go to:

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www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)