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Homocysteine Lyase Activity Assay Kit (Fluorometric)

11/19

(Catalog # K2026-100; 100 assays; Store at -20°C)

I. Introduction:

Homocysteine α,γ-Lyase (HCYase) is a pyridoxal 5'-phosphate dependent enzyme that catalyzes the breakdown of homocysteine to hydrogen sulfide, ammonia, and α-ketobutyrate. HCYase is present in high activity in the anaerobic protozoan parasite *Trichomonas vaginalis* but is absent from mammals. The *Trichomonas* HCYase is highly selective for homocysteine as compared to cysteine. Additionally, it has broad pH stability (pH 6-8) and is thermally stable up to 50°C. This makes rHCYase a promising candidate for therapeutic applications including the diagnosis of hyperhomocystenemia, which has been demonstrated to be a major risk factor in cardiovascular diseases. **BioVision's Homocysteine Lyase Activity Assay Kit** is a simple, quick and sensitive method to assay HCYase activity in bacterial samples. The assay relies on the HCYase-catalyzed release of hydrogen sulfide (H_2S), which reacts with a fluorogenic probe to form a stable fluorophore measured at Ex/Em = 368/460 nm.

II. Application:

- Determining the activity of wild type or recombinant Homocysteine α,γ-Lyase

III. Sample Type:

- Bacterial cell lysate

IV. Kit Contents:

Components	K2026-100	Cap Code	Part Number
HCY Assay Buffer	25 ml	WM	K2026-100-1
HCY Substrate	5 vials	Red	K2026-100-2
HCY Probe	500 µl	Violet	K2026-100-3
AMC Standard	100 µl	Yellow	K2026-100-4
Positive Control	1 vial	Green	K2026-100-5

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom (low/medium binding)
- Multi-well spectrophotometer (Fluorescent plate reader)
- Bacterial lysis buffer

VI. Storage Conditions and Reagent Preparation:

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

- **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.

VII. Homocysteine Lyase Activity Assay Protocol:

1. Sample Preparation: Grow bacteria according to the experimental conditions. Harvest the bacterial cells by centrifugation. 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. Dilute the lysate to 1:50 dilution using HCY Assay Buffer. 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.
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. For **Background Control [BC]** well, add 100 µl of HCY Assay Buffer.
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 -80ºC 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.

2. Standard Curve Preparation: Prepare 10 fold dilution of the AMC Standard by adding 10 µl of the AMC Standard with 90 µl of HCY Assay Buffer. 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. 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.

3. Reaction Mix Preparation: 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 & BC wells, prepare 50 µl Reaction Mix containing:

	Reaction Mix
HCY Assay Buffer	46 µl
HCY Probe	4 µl

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.

4. 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.

5. Calculation: Subtract the 0 Standard RFU readings from all Standard RFU readings. Plot the AMC Standard Curve. For each Sample type, choose any two time points within the linear range of the curve (t_1 & t_2). 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 & t_2 time points: **[F = RFU_{Sample} – RFU_{BC}]**. Apply the net fluorescence signal to the AMC Standard Curve to get **B** pmol of AMC generated during the reaction time ($\Delta t = t_2 - t_1$).

Calculate the Homocysteine Lyase activity in Samples using the following equation:

$$\textbf{Homocysteine Lyase Activity} = \frac{B}{V*\Delta T} \times D = \textbf{mU/ml}$$

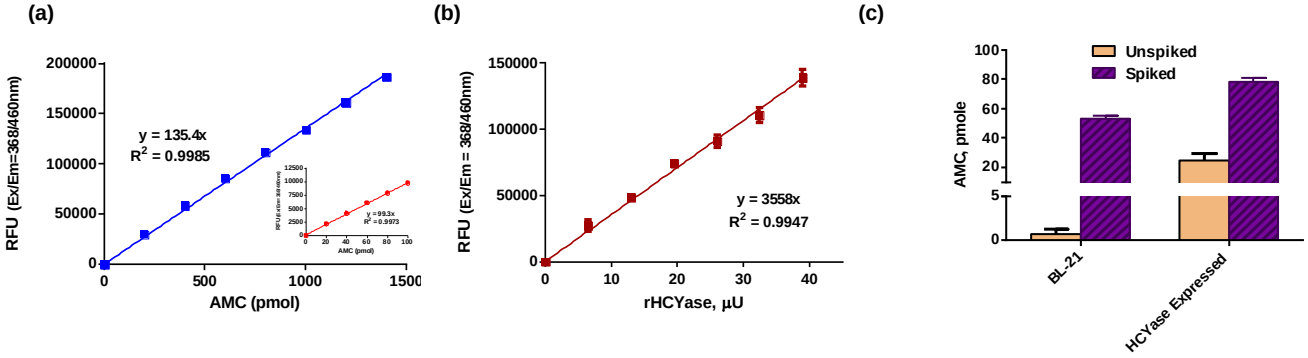
Where **B** is the amount of AMC calculated from the AMC Standard Curve (pmol)

V is the volume of sample added to the well (ml)

Δ**T** is the time between t_2 and t_1 in minutes

D is the 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.



Figures: (a) AMC Standard Curve. **(b)** Activity of rHCYase. **(c)** Estimation of HCYase activity in lysates of wild type *E. coli* (BL-21) and rHCY expressing *E. coli*. Both lysates were spiked with 1.6 µU of Positive Control. The average spiked recoveries were 90.9% and 104.1% respectively. The assay was performed according to the kit assay protocol.

VIII. Related Products:

Cystathionine β Synthase, human recombinant (Cat. # 7844-100, 500)
Cystathionine β Synthase Activity Assay Kit (Fluorometric) (Cat. # K998-100)
Cysteine Assay Kit (Fluorometric) (Cat. # K558-100)
Homocysteine α,γ-Lyase (rHCYase), Active, Recombinant (Cat. # P1117-200)
Homocysteine Assay Kit (Fluorometric) (Cat. # K531-100)
Homocysteine ELISA Kit (Cat. # E4543 -100)
Methionine Assay Kit (Fluorometric) (Cat. # K442-100)
L-Methionine γ-Lyase, *Pseudomonas putida* recombinant (Cat. # 7848-100, 500)

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