Tribioscience

Homocysteine Fluorometric Assay (TBS2091, 100 Assays, Store at -20 °C)

DESCRIPTION

Homocysteine (Hyc) is a sulfur amino acid. It exists mainly as an oxidized disulfide form in circling system. The reduced (free) homocysteine can be metabolized into cysteine. Homocysteine is highly related to cardiovascular disease, ischemic stroke and myocardial infarction. Elevated homocysteine has been linked to cardiovascular disease, cognitive decline, and pregnancy complications.

Tribioscience's Homocysteine Assay measures homocysteine concentrations in plasma or serum and other bio-samples with coupling enzyme reactions. The resulting fluorescent signal at Ex/Em = 400/465 nm is in proportion to homocysteine concentrations in samples. The kit provides the easiest and most accurate approach to measuring homocysteine levels in a sample.

SYNONYMS: Hcy, Hocy, tHcy.

APPLICATIONS

Measure Homocysteine in a variety of samples.

KIT CONTENTS FOR 100 TESTS:

Name	Size (100 tests)
Hcy Assay Buffer	10 mL
Hcy Probe (50X)	25 µL
Homocysteine Standard (2 mM)	100 µL
Hcy Enzyme I	110 µL
Hcy Enzyme II	60 µL
Hcy Co-factor	30 µL
Reducing Agent	60 µL
Hcy Substrate	300 µL

Storage conditions: Store the Reagent at –20°C, protected from light. Shelf life: 12 months.

PROCEDURES

1. Equilibrate all the kit components until room temperature before starting the experiment.
2. Prepare the homocysteine standard as Table 1. Label tube from 1 to 8. Add 470 µL Assay buffer in tube I and 200 µL assay buffer from tube 2 to tube8.
3. Making 100 µM of Hcy by adding 25 µL of 2 mM Hcy and adding 5 µL of Reducing Agent into Tube #1, then make a 2-fold sequential dilution by transfer 200 µL of higher concentration to the next tube through tube 7. Tube # 8 as blank.

Table 1: Homocysteine Standard preparation

Tube #	Vol. Hcy Standard	Assay Buffer (µL)	Hcy concentration (µM)	Hcy (pmol/Well)
1	25µL 2 mM Hcy +5 µL Reducing Agent	470	100	5000
2	200 µL of tube 1	200	50	2500
3	200 µL of tube 2	200	25	1250
4	200 µL of tube 3	200	12.5	625
5	200 µL of tube 4	200	6.25	312.5
6	200 µL of tube 5	200	3.12	156.25
7	200 µL of tube 6	200	1.56	78.125
8	0	200	0	0

4. For test samples, 100 µL of sample by adding 1 µL of Reducing Agent.
5. Add 50 µL of Hcy standards or 50 µL of test sample to indicate wells in duplicate manner.
6. Cover the plate and incubate for 30 minutes at 37ºC by gently shaking.
7. Prepare working solution mixture for 100 tests:
   250 µL of substrate + 100 µL of enzyme I + 50 µL of enzyme II + 25 µL of co-factor + 3575 µL of assay buffer. This mixture is enough for 100 tests.
8. Adding 40 µL of working solution mixture to each well.
9. Cover the plate and incubate for 60 minutes at 37ºC by gently shaking.
10. Prepare Hcy Probe working solution: add 20 µL of Hcy Probe (50X) to 980 µL of DMSO for 100 wells.
11. Add 10 µL of the Hcy Probe working solution to the test samples and Hcy standards.
12. Incubate at 37°C for 10 minutes with gentle shaking and protected from light.
13. Read the plate at excitation and emission wavelength at Ex/Em = 400 nm/465 nm respectively.
14. Calculate the Homocysteine concentration by the typical Hcy standard curve as follows:
    Y=A*X + B
    Homocysteine (pmol/well): X = DF *((Y -B)/ A))

Here: **Y= RFU; A=Slope; B=intercept; X= Free Thiol product (pmol); DF=dilution factor**

TYPICAL DATA

This standard curve is provided for demonstration only as below **Fig.1** and **Fig.2** A standard curve should be generated for each set of samples assayed.

Fig.1: Homocysteine Standard Curve

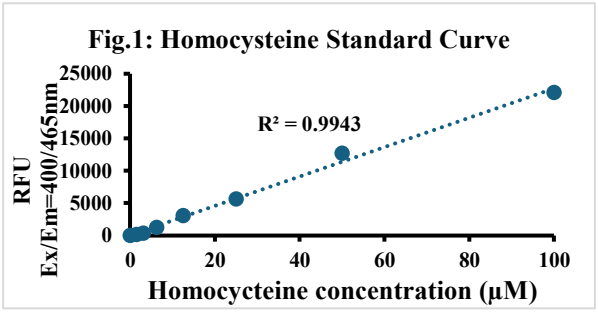
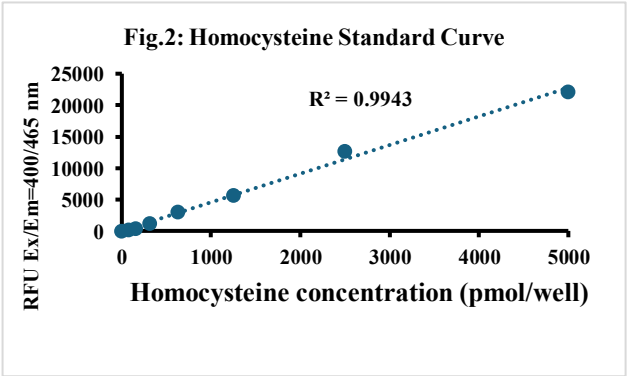


Fig.2: Homocysteine Standard Curve



RELATIVE PRODUCTS

Resazurin Cell Viability Kit (TBS2001)
ATP Colorimetric/Fluorometric Assay (TBS2010)
ADP Colorimetric/Fluorometric Assay Kit (TBS2020)
CCK-8 Cell Viability Assay (TBS2022)
Thiol Fluorometric Assay (TBS2026)
GSH Assay (TBS2028)
Caspase-3 Colorimetric Assay kit (TBS2030)
AHCY Activity Fluorometric Assay (TBS2056)
Glucose Oxidase Colorimetric/Fluorometric Assay (TBS2088)
NNMT Inhibitor Screening Assay (TBS2097)
NNMT Activity Fluorometric Assay (TBS2098)
G6PDH Activity Colorimetric Assay (TBS2102)
AHCY Inhibitor Screening Fluorometric Assay (TBS2099)
GOT Activity Assay (TBS2013)

This product is for research use only.

 365 San Aleso Ave, Sunnyvale, CA 94085, USA Website: www.tribioscience.com
Tel: 408-498-0197/ 833-697-8998 Email: order@tribioscience.com