Technical Bulletin

HMG-CoA Reductase Assay Kit

Catalogue number CS1090

Product Description

3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) is a transmembrane glycoprotein, located on the endoplasmic reticulum.[1] This enzyme catalyzes the four-electron reduction of HMG-CoA to coenzyme A (CoA) and mevalonate, which is the rate-limiting step in sterol biosynthesis.[2] The activity of HMGR is controlled through synthesis, degradation, and phosphorylation in order to maintain the concentration of mevalonate derived products. In addition to the physiological regulation of HMGR, the human enzyme has been targeted successfully by drugs in the clinical treatment of high serum cholesterol levels.[3,4] Controlling serum cholesterol levels has an important therapeutic role as hypercholesterolemia often leads to the development of atherosclerosis and consequently to cardiovascular pathologies, which might result in myocardial infarction and stroke. Recent evidence suggests that a disturbance of cholesterol homeostasis contributes to the development of a chronic inflammatory state.[5]

Reaction scheme of HMGR:

$$\text{HMG-CoA} + 2\text{NADPH} + 2\text{H}^+ \rightarrow \text{mevalonate} + 2\text{NADP}^+ + \text{CoA-SH}$$

The HMGR Assay Kit is an important tool for the basic research of cholesterol and other related metabolic pathways. The kit is designed for the detection of HMGR activity. A major function of this kit is to screen for different inhibitors/activators of the purified catalytic subunit of the enzyme, which may play a crucial role in therapeutics. The assay is based on the spectrophoto-metric measurement of the decrease in absorbance at 340 nm, which represents the oxidation of NADPH by the catalytic subunit of HMGR in the presence of the substrate HMG-CoA.

Components

The kit is sufficient for 30 assays of 1 ml or 100 assays of 200 µl

- Assay Buffer, 5x — 10mL
  Catalog Number A5981
- NADPH — 25mg
  Catalog Number N6505
- Substrate Solution (HMG-CoA) — 2mL
  Catalog Number S7447
- HMG-CoA Reductase (catalytic domain) — 200µl
  0.50–0.70 mg/ml
  Catalog Number H8789
- Inhibitor Solution (Pravastatin) — 200µl
  Catalog Number I5909

Equipment Required but Not Provided

- 1 ml spectrophotometer cuvette (quartz, Catalog Number Z600091) or UV 96 well plate (Catalog Number CLS3635)
- UV/Vis Spectrophotometer

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on dry ice and storage at –70 °C is recommended. Upon first use, store the components in working aliquots.

Preparation Instructions

Reagent Preparation

Use ultrapure water (17 MΩ-cm or equivalent) for the preparation of reagents and throughout the procedure.

1x Assay Buffer - Dilute the Assay buffer, 5x (Catalog Number A5981) 5-fold with ultrapure water (For example- dilute 0.2 mL of Assay buffer, 5x with 0.8 mL of ultrapure water). 1 mL of 1x Assay Buffer is sufficient for 1 sample in a 1 mL cuvette assay or 5 samples using a 96 well plate. Keep on ice or store at –20 °C for further use. Keep the concentrated Assay Buffer, 5x in working aliquots at –20 °C.

NADPH Reconstitution - Reconstitute the NADPH (Catalog Number N6505) with 1.5 mL of 1x Assay Buffer. Mix well and store in working aliquots at –20 °C.

Procedure

The enzyme and the substrate are less stable when added to the assay buffer. Hence, it is very important to add the different components according to the order mentioned in this procedure in order to obtain best results.

Thaw an aliquot of the HMG-CoA Reductase (HMGR) on ice and keep it cold throughout the procedure. Try not to keep it on ice for more than 60 minutes, since longer storage can result in reduction of enzyme activity.

All the other kit components can be thawed at room temperature and once thawed, should be kept on ice.

1. Before beginning, set the spectrophotometer at 37 °C and 340 nm, with a kinetic program:

   1 mL Sample: read every 15 seconds for up to 5 minutes.

   96 well plate Sample: read every 20 seconds for up to 10 minutes.
2. Add appropriate volumes of the reaction solutions according to Table 1 (1 mL assay) or 2 (96 well plate).
3. Equilibrate the Assay buffer to 37 °C before adding the adding the other components

Table 1
Reaction volumes for 1 mL Samples

Sample	1× Assay buffer	Pravastatin	NADPH	HMG-CoA	HMGR
Blank	920 µl	–	20 µl	60 µl	–
Activity	915 µl	–	20 µl	60 µl	5 µl
Inhibition	910 µl	5 µl	20 µl	60 µl	5 µl

Table 2
Reaction volumes for 96 well plate Samples

Sample	1x Assay buffer	Pravastatin	NADPH	HMG-CoA	HMGR
Blank	184 µl	–	4 µl	12 µl	–
Activity	182 µl	–	4 µl	12 µl	2 µl
Inhibition	181 µl	1 µl	4 µl	12 µl	2 µl

4. Add the reagents to the reaction (wells or cuvette) in the following order:

   4.1 Add the 1x Assay buffer to all Samples.
   4.2 Add the inhibitor (Pravastatin) to the inhibition sample.
   4.3 Add the reconstituted NADPH to all Samples.
   4.4 Add Substrate Solution (HMG-CoA) to all Samples.
   4.5 Add HMG-CoA Reductase (HMGR) to the Activity and Inhibition Samples.
   4.6 Mix the samples thoroughly.

   Note: When using a plate reader, shake the plate vigorously for at least 10 seconds before the first absorbance measurement.
5. Start the kinetics program immediately. The A_{340} will decrease due to the decrease in NADPH concentration.

Results

Specific activity calculation

For an accurate calculation it is recommended to perform the assay in 1 mL samples.

Calculate the activity of the product according to the following equation:

$$\text{Units/mgP} = \frac{(\Delta A_{340}/\text{min}_{\text{sample}} - \Delta A_{340}/\text{min}_{\text{blank}} \times TV}{12.44 \times V \times 0.6 \times LP}$$

12.44 = ε^{mM} - the extinction coefficient for NADPH at 340 nm is 6.22 $mM^{-1}cm^{-1}$. 12.44 represents the 2 NADPH consumed in the reaction.
TV = Total volume of the reaction in ml (1 mL for cuvettes and 0.2 mL for plates)

V = volume of enzyme used in the assay (mL)

0.6 = Enzyme concentration in mg-protein (mgP)/mL (0.50–0.70 mgP/mL)

LP = Light path in cm
(1 for cuvettes and 0.55 for plates)

Unit definition: One unit will convert 1.0 µmole of NADPH to $NADP^+$ per 1 minute at 37 °C. The unit specific activity is defined as µmol/min/mg-protein (Units/mgP).

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cs1090pis Rev 06/23