

3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase (HMGR) Assay Kit

Catalog No.: abx294445

Size: 96 tests

Detection Range: 0.67 U/L – 24.65 U/L

Sensitivity: 0.67 U/L

Storage: Store all components at -20°C in the dark.

Application: For detection and quantification of HMGR activity in animal tissue homogenates.

Principle of the Assay

Abbexa's 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase (HMGR) Assay Kit is a quick, convenient, and sensitive method for measuring and calculating HMGR activity. HMGR catalyzes the conversion of HMG-CoA, consuming NADPH during the reaction and resulting in a decrease in absorbance at 340 nm. The rate of absorbance decline is directly proportional to HMGR activity, which can then be calculated.

Kit components

1. 96-well UV-microplate
2. Extraction Solution A: 2 × 60 ml
3. Extraction Solution B: 1.2 ml
4. Buffer Solution: 20 ml
5. Acceleration Reagent: 4 vials
6. Substrate: 0.24 ml
7. Plate Sealer: 2

Materials required but not provided

1. Microplate reader (340 nm)
2. Double-distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. Centrifuge and centrifuge tubes
6. Vortex mixer
7. Mechanical homogenizer
8. Incubator
9. Vortex mixer

Protocol

A. Preparation of samples and reagents

1. Reagents

- **Extraction Working Solution:** Prepare enough Extraction Working Solution for the wells tested. Mix Extraction Solution A and Extraction Solution B in a ratio of 99 : 1. For example, prepare 500 µl of Extraction Working Solution by mixing 495 µl of Extraction Solution A and 5 µl of Extraction Solution B. Unused Extraction Working Solution can be kept on ice for up to 1 day in the dark.
- **Acceleration Reagent Working Solution:** Reconstitute 1 vial of Accelerant Reagent with 0.72 ml of double-distilled water. Mix thoroughly until fully dissolved. Unused Acceleration Reagent Working Solution can be stored at -20°C for up to 3 days in the dark.
- **Substrate Working Solution:** Prepare enough Substrate Working Solution for the wells tested. Mix Substrate and Buffer Solution in a ratio of 1 : 9. For example, prepare 100 µl of Substrate Working Solution by mixing 10 µl of Substrate and 90 µl of Buffer Solution. Unused Substrate Working Solution can be kept on ice for up to 1 day in the dark.
- **Reaction Working Solution:** Prepare enough Reaction Working Solution for the wells tested. Mix Acceleration Reagent Working Solution, Substrate Working Solution and Buffer Solution in a ratio of 1 : 1 : 7. For example, prepare 450 µl of Reaction Working Solution by mixing 50 µl of Acceleration Reagent Working Solution, 50 µl of Substrate Working Solution, and 350 µl of Buffer Solution. Unused Substrate Working Solution can be kept on ice for up to 1 day in the dark. Before use, keep the Reaction Working Solution at 25°C for at least 5 minutes. Unused Reaction Working Solution can be kept on ice for up to 1 day in the dark.

Note:

- Allow all reagents to equilibrate to room temperature before use.

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Carefully weigh at least 20 mg of tissue. Wash tissue with cold PBS (0.01M, pH 7.4). Per 20 mg of tissue, add 180 µl of Extraction Working Solution. Homogenize manually or use a mechanical homogenizer at 4°C. Centrifuge at 10,000 x g for 10 min at 4°C. Carefully take the supernatant for detection. Keep on ice and assay immediately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Extraction Working Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

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Sample Type	Dilution Factor
10% Mouse liver tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse heart tissue homogenate	1
10% Mouse calf muscle tissue homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

B. Assay Procedure

1. Mark positions on the 96-well UV-microplate for each blank and sample. Each sample requires a corresponding control. It is strongly recommended to test all wells in duplicate.
2. Add 20 µl of Buffer Solution to the blank wells.
3. Add 20 µl of the samples to the sample well.
4. Add 180 µl of Reaction Working Solution to all wells.
5. Mix the well contents by tapping the plate, or shaking with a microplate shaker, for at least 5 seconds
6. Measure the OD value of each well with a microplate reader at 340 nm. Record this value as A₁.
7. Cover with a plate sealer, then incubate at 25°C for 20 minutes.
8. Unseal the plate, then measure the OD value of each well with a microplate reader at 340 nm. Record this value as A₂.

C. Calculation of Results

For each sample, calculate the difference in absorbance between the two measurements: $\Delta OD = A_1 - A_2$

One unit of HMGR activity is defined as the quantity of HMGR in 1 kg of tissue that catalyzes the consumption of 1 µmol of NADPH in one minute at 25°C.

$$\text{HGMR activity} \left(\frac{U}{kg \text{ wet weight}} \right) = \frac{(\Delta OD_{\text{Sample}} - \Delta OD_{\text{Blank}}) \times \epsilon \times V_{\text{total}}}{t \times 0.6 \times \epsilon \times m \times V_{\text{sample}}} \times V \times f$$

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where:

$\Delta OD_{\text{Sample}}$	$A_1 - A_2$ value of the sample.
ΔOD_{Blank}	$A_1 - A_2$ value of the blank
t	Reaction time, 20 minutes
0.6	Optical path of microplate wells, cm
ϵ	Molar extinction coefficient at 340 nm, $16.22 \times 10^{-3} \text{ L}/\mu\text{mol}\cdot\text{cm}$
V_{total}	Volume of reaction system, 0.2 ml
V_{sample}	Volume of sample, 0.02 ml
m	Weight of tissue, kg
V	Volume of extraction solution, L
f	Dilution factor of sample before test

Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.