ab204701
HMG-CoA Reductase Activity Assay Kit (Colorimetric)

Instructions for Use

For rapid, sensitive and accurate detection of HMG-CoA Reductase Activity.

View kit datasheet: www.abcam.com/ab204701
(use www.abcam.cn/ab204701 for China, or www.abcam.co.jp/ab204701 for Japan)

This product is for research use only and is not intended for diagnostic use.

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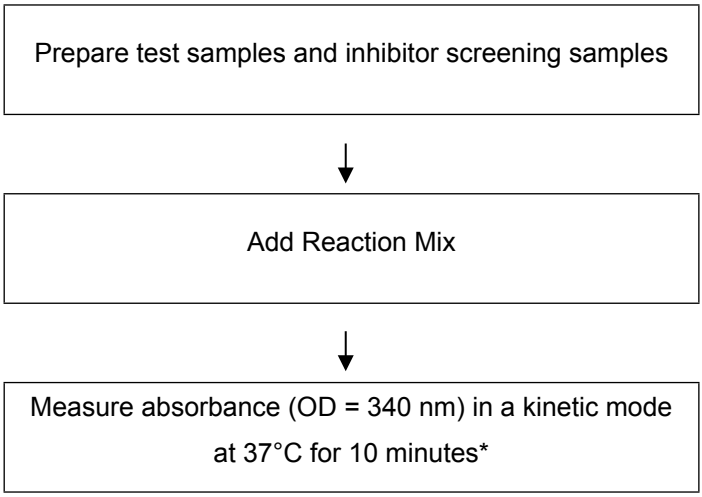
1. BACKGROUND

HMG-CoA Reductase Activity Assay Kit (Colorimetric) (ab204701) is suitable for measuring activity of purified HMG-CoA reductase or for screening inhibitors/activators of HMG-CoA reductase. It is based on the consumption of NADPH by the enzyme, which can be measured by the decrease of absorbance at OD=340 nm. The limit of detection is below 0.05 mU.

HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA reductase or HMGR) (EC 1.1.1.34) is the rate-controlling enzyme of the mevalonate pathway, the metabolic pathway that produces cholesterol from acetyl-CoA. In an NADPH-dependent reaction, HMG-CoA reductase reduces HMG-CoA to generate mevalonate and CoA. The enzyme is target of a group of cholesterol-lowering drugs known as statins. Inhibition of HMG-CoA reductase induces expression of LDL receptors in the liver, which lowers plasma concentration of cholesterol.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -80ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
HMG-CoA Reductase Assay Buffer	20 mL	-80°C	-80°C
HMG-CoA Reductase	1 Vial	-80°C	-80°C
HMG-CoA	1 Vial	-80°C	-80°C
NADPH	1 Vial	-80°C	-80°C
Inhibitor (Atorvastatin, 10 mM)	10 µL	-80°C	-80°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD = 340 nm
- 96 well plate with clear flat bottom
- Heat block or water bath

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **HMG-CoA Reductase Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -80°C.

9.2 **HMG-CoA Reductase:**

Reconstitute enzyme (0.7 mg/mL) in 550 µL of HMG-CoA Reductase Assay Buffer. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store at -80°C. Keep on ice while in use.

9.3 **HMG-CoA:**

Reconstitute HMG-CoA in 1.3 mL ddH_2O, make sure the material is completely dissolved. Aliquot HMG-CoA so that you have enough volume to perform the desired number of assays. Store at -80°C. Keep on ice while in use.

9.4 **NADPH:**

Reconstitute NADPH in 440 µL ddH_2O, make sure the material is completely dissolved. Aliquot NADPH so that you have enough volume to perform the desired number of assays. Store at -80°C. Keep on ice while in use.

9.5 **Inhibitor (Atorvastatin, 10 mM):**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot atorvastatin so that you have enough volume to perform the desired number of assays. Store at -80°C.

10. SAMPLE PREPARATION

10.1 **Purified protein:**

Purified protein can be used directly.

To find optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample.

10.2 **Screening Compounds:**

Dissolve test compounds into appropriate solvent.

11. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**

11.1 **Set up Reaction wells:**

- Sample wells = 0.5 – 15 mU enzyme (adjust volume to 10 µL/well with Assay Buffer).
- Positive control well = 1 – 5 µL HMG-CoA Reductase (adjust volume to 10 µL/well with Assay Buffer).
- Inhibitor Control well = 5 µL HMG-CoA Reductase + 2 µL of Inhibitor (adjust volume to 10 µL with Assay Buffer).
- Reagent Background control well = 10 µL HMG-CoA Reductase Assay Buffer.

11.2 **OPTIONAL – Inhibitor Screening:**

- Dissolve test inhibitor to 100X in an appropriate solvent.
- Test inhibitor wells = Add 2 µL of test inhibitor + 5 µL of provided HMG-CoA Reductase (adjust volume to 10 µL with Assay Buffer).
- Enzyme Control (EC) well = 5 µL of reconstituted HMG-CoA Reductase (adjust volume to 10 µL with Assay Buffer).
- Solvent Control well = 2 µL solvent + 5 µL of provided HMG-CoA Reductase (adjust volume to 10 µL with Assay Buffer). ***NOTE:*** *Inhibitor provided in the kit and inhibitors dissolved in DMSO do not require a solvent control.*

11.3 **Reaction Mix:**

Prepare 190 µL of Reaction Mix for each reaction:

Component	Reaction Mix (µL)
HMG-CoA	**12**
NADPH	**4**
HMG-CoA Reductase Assay Buffer	**174**

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1).

11.4 Add 190 µL of Reaction Mix into each well.

11.5 Mix well.

11.6 Immediately measure output on a colorimetric microplate reader at OD = 340 nm in a kinetic mode, every 2 – 3 minutes, for at least 10 minutes at 37°C protected from light.

NOTE: *Sample incubation time can vary depending on HMG-CoA Reductase activity in the samples. We recommend measuring absorbance in kinetic mode and then choosing two time points (T_1 and T_2) during the linear range.*

OD value at T_2 should not exceed the highest OD in the standard curve. For standard curve, do not subtract A_1 from A_2 reading.

12. CALCULATIONS

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

12.1 Take the absorbance (A_{340nm1} and A_{340nm2}) at two time points (T_1 and T_2) in the linear range. Readings should be at least 2 minutes apart.

12.2 To determine Activity, use the following equation:

$$HMG - CoA\ Reductase\ Activity$$

$$= \frac{(\frac{-\Delta A_{340nm}}{\Delta T}\ Test - \frac{-\Delta A_{340nm}}{\Delta T}\ Reagent\ Background)\ x\ (0.2)}{(12.44)\ x\ V\ x\ P\ x\ (0.55)}$$

$$= Units/mg\ protein$$

Where:

0.2 = Reaction volume (mL)

12.44= millimolar extinction coefficient of NADPH x 2 (2 NADPH consumed in each reaction)

V = Enzyme volume (mL)

P = Initial enzyme concentration in mg-protein/ml (mgP/ml)

0.55 = light path (cm)

12.3 For inhibitor screening, calculate percent inhibition using the following equation:

$$\%\ Inhibition$$

$$= \frac{\frac{-\Delta A_{340nm}}{\Delta T}(Enzyme) - \frac{-\Delta A_{340nm}}{\Delta T}(Enzyme + Inhibitor)}{\frac{-\Delta A_{340nm}}{\Delta T}(Enzyme)} * 100$$

Unit Definition:

1 Unit HMG-CoA Reductase activity = amount of enzyme that converts 1.0 µmol of NADPH to $NADP^+$ per min. at pH 7.5 at 37°C.

13. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

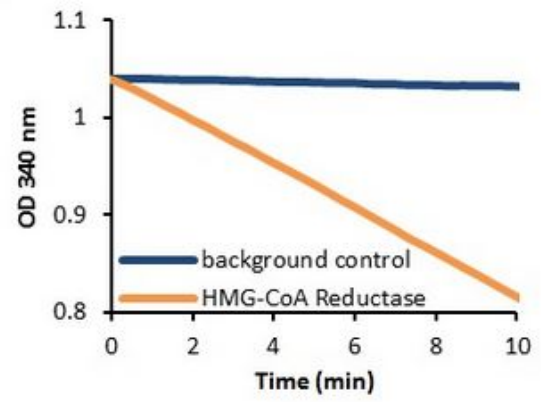


Figure 1. Activity of HMG-CoA Reductase (positive control included in the kit) compared to the background control with no enzyme.

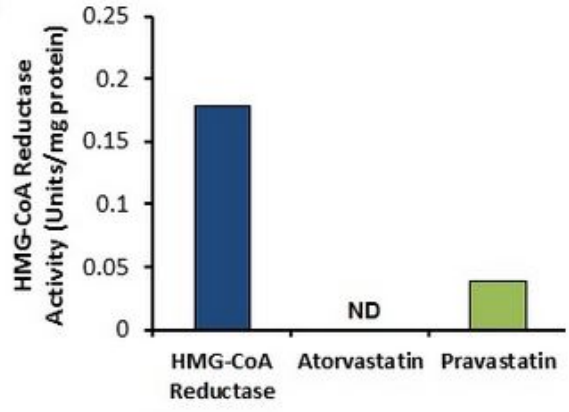


Figure 2 Comparison of HMG-COA Reductase activity in absence or presence of specific inhibitors such as atorvastatin (100 µM) and pravastatin (10 ng/µl); *ND: Not Detectable*.

14. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare test samples and inhibitor screening samples in duplicate
- Prepare Reaction Mix (Number samples + inhibitors + controls + 1).

Component	Reaction Mix (µL)
HMG-CoA	**12**
NADPH	**4**
HMG-CoA Reductase Assay Buffer	**174**

- Add 190 µL of Reaction Mix to the test sample, control and inhibitor screening sample wells.
- Incubate plate at 37°C during 10 minutes and read absorbance at OD = 340 nm in a kinetic mode.

15. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Clear plates
Sample with erratic readings	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

16.FAQ

17.NOTES

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