



HMG-CoA Reductase Activity/Inhibitor Screening Kit (Colorimetric)

rev 08/19

(Catalog # K588-100; 100 assays; Store at -80°C)

I. Introduction:

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. BioVision's HMG-CoA Reductase Activity Assay Kit measures utilization of NADPH, which can be measured by the decrease of absorbance (OD 340 nm). The kit is suitable for measuring activity of purified enzyme and screening inhibitors/activators of HMG-CoA reductase. The limit of detection is below 0.05 mU.

II. Application:

- Measurement of HMG-CoA reductase activity
- Screening/studying/characterizing HMG-CoA reductase inhibitors/activators

III. Sample Type:

- Partially purified and recombinant enzyme

IV. Kit Contents:

Components	K588-100	Cap Code	Part Number
HMG-CoA Reductase Assay Buffer	20 ml	WM	K588-100-1
HMG-CoA Reductase	1 Vial	Green	K588-100-2
HMG-CoA	1 Vial	Red	K588-100-3
NADPH	1 Vial	Blue	K588-100-4
Inhibitor (Atorvastatin, 10 mM)	10 µl	Yellow	K588-100-5

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage Conditions and Reagent Preparation:

Store kit at -80°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **HMG-CoA Reductase Assay Buffer:** Pre-warm buffer to 37°C before use.
- **HMG-CoA Reductase:** Reconstitute in 550 µl of HMG-CoA Reductase Assay Buffer. Aliquot and store at -80°C. Avoid repeated freeze/thaw cycles. Keep on ice during use.
- **HMG-CoA:** Reconstitute in 1.3 ml dH₂O, make sure the material is completely dissolved. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use.
- **NADPH:** Reconstitute in 440 µl dH₂O, make sure the material is completely dissolved. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use.
- **Inhibitor (Atorvastatin, 10 mM):** Bring to room temperature before use.

VII. HMG-CoA Reductase Activity Assay Protocol:

1. **Sample Preparation:** To test sample HMG-CoA reductase, add 0.5-15 mU enzyme into desired well(s) in a 96-well plate. For positive control, add 1-5 µl of provided HMG-CoA Reductase into desired well. For Inhibitor Control, add 5 µl of HMG-CoA Reductase and 2 µl of Inhibitor into one of the wells. Adjust the volume of sample, positive control and inhibitor control wells to 10 µl per well with HMG-CoA Reductase Assay Buffer. For reagent background control, add 10 µl HMG-CoA Reductase Assay Buffer.

Note:

For samples with unknown HMG-CoA reductase activity, we suggest testing several amounts of enzyme to ensure the activity is within the assay range.

2. **Inhibitor Screening (optional):** To test HMG-CoA reductase inhibitors, dissolve test inhibitor to 100X in an appropriate solvent. Add 2 µl of test inhibitor and 5 µl of provided HMG-CoA Reductase into Test Inhibitor well(s). Prepare a parallel well as Enzyme Control (EC) by adding 5 µl of provided HMG-CoA Reductase. Adjust the volume of Test Inhibitor and Enzyme Control wells to 10 µl/well with HMG-CoA Reductase Assay Buffer.

Note:

Prepare a solvent control well by adding 2 µl of solvent as the Test Inhibitor to test its effect on HMG-CoA reductase activity. Adjust the volume to 10 µl with HMG-CoA Reductase Assay Buffer. Provided Inhibitor and inhibitors dissolved in DMSO do not require a solvent control.

3. **Reaction Mix:** Prepare enough reaction mix for the number of wells (test HMG-CoA Reductase, positive control, inhibitor control, reagent background control, Test Inhibitor, and Enzyme Control) to be analyzed. For each well, prepare 190 µl reaction mix:

	Reaction Mix
HMG-CoA	12 µl
NADPH	4 µl
HMG-CoA Reductase Assay Buffer	174 µl



Mix and add 190 µl of the reaction mix into each well, mix well.

- Measurement:** Immediately measure absorbance kinetically at OD 340 nm in a microplate reader at 37°C for 10 min.
- Calculation:** 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 min. apart.

To determine Activity, use the following equation:

$$\text{Sample HMG-CoA Reductase Activity} = \frac{\left(\frac{-\Delta A_{340nm} \text{Test}}{\Delta T} - \frac{-\Delta A_{340nm} \text{Reagent Background}}{\Delta T} \right) \times (0.2)}{(12.44) \times V \times P \times (0.55)} = \text{Units/mg protein}$$

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)

For inhibitor screen, calculate percent inhibition using the following equation:

$$\% \text{ Inhibition} = \frac{\frac{-\Delta A_{340nm}(\text{Enzyme})}{\Delta T} - \frac{-\Delta A_{340nm}(\text{Enzyme} + \text{Inhibitor})}{\Delta T}}{\frac{-\Delta A_{340nm}(\text{Enzyme})}{\Delta T}} \times 100$$

Unit Definition: One unit of HMG-CoA Reductase is the amount of enzyme that converts 1.0 µmol of NADPH to NADP⁺ per min. at pH 7.5 at 37°C.

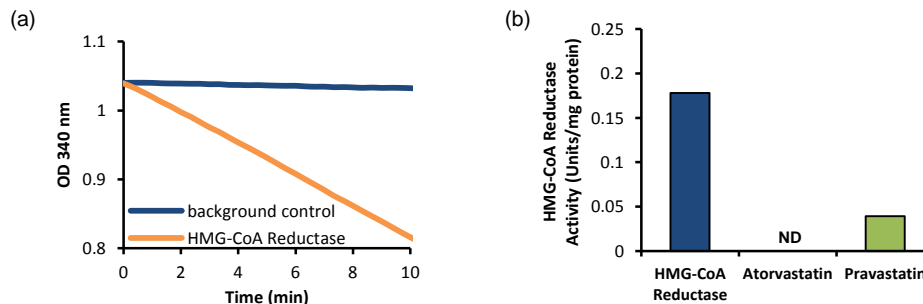


Figure: (a) HMG-CoA Reductase (Positive Control) activity. (b) Inhibition of HMG-CoA Reductase activity by Atorvastatin (100 µM) and Pravastatin (10 ng/µl). ND: Not Detectable. Assay was performed according to kit protocol.

VIII. RELATED PRODUCTS:

Coenzyme A (CoA) Colorimetric/Fluorometric Assay Kit (K367)
Total Cholesterol and Cholesteryl Ester Assay Kit (K603)
Total Cholesterol and Cholesteryl Ester Assay Kit II (K623)
HMG-CoA Reductase Antibody (3952)
Atorvastatin (2278)
Rosuvastatin (1955)
Simvastatin (1693)

Picoprobe™ Acetyl-CoA Fluorometric Assay Kit (K317)
Cholesterol Efflux Fluorometric Assay Kit (K582)
NADPH (2738)
HMG-CoA Blocking Peptide (3952BP)
Lovastatin (1692)
Mevastatin (1694)
Naringenin (1772)

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