BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

Department of Biochemistry, Attn: Dr. Lee, University at Buffalo, 955 Main St., Suite 4102, Buffalo, NY 14203
Tel: (716) 4586903 Email: chunglee@buffalo.edu Web: www.bmrservice.com

Thiolase II (Acetyl-CoA acetyltransferase) Assay Kit (Cat #: E-166)

COMPONENTS: Thiolase II Assay Solution- 5 ml (for 100 wells), store at -80°C **(shield solution from light during use)**
10x Acetyl-CoA- 0.5 ml, store at -80°C **(shield solution from light during use)**
10x Cell Lysis Solution- 25 ml, store at room temperature (RT)

PRODUCT DESCRIPTION: The thiolase II activity assay is based on coenzyme A (CoA)-dependent reduction of iodonitrotetrazolium (INT) to formazan, which exhibits an absorption maximum at 492 nm (ε = 18 $mM^{-1}cm^{-1}$) and allows for measurement of thiolase II activity in cell/tissue lysates. Reagents are stable for at least one year if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with ice-cold dH_2O. Bring up ~10^5 washed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (use ~5 mg tissue in 0.5 ml).

2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.

3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.5 – 4 mg/ml. *Normalization of sample protein concentration is recommended.*

Reagent thawing:

Keep thawed Thiolase II Assay Solution and 10x Acetyl-CoA on ice and shielded from light. Do not over thaw. Freeze solutions immediately after use.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of dH_2O and 10 parts of Thiolase II Assay Solution, e.g., 50 µl dH_2O + 500 µl Thiolase II Assay Solution. Keep solution on ice shielded from light.

Reaction solution is prepared by mixing 1 part of 10x Acetyl-CoA and 10 parts of Thiolase II Assay Solution, e.g., 50 µl 10x Acetyl-CoA + 500 µl Thiolase II Assay Solution. Keep solution on ice shielded from light.

Enzyme assay:

1. Thaw lysates quickly and keep on ice. *Do not over thaw.* Add 20 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate. For drug discovery application, mix 1 µl of an inhibitor with sample in wells first.

2. After all samples are pipetted to the plate, add 50 µl control solution to one set of sample wells and 50 µl reaction solution to the other set of sample wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C humidified incubator for 90 min and 120 min. *Do not use CO_2 incubator.*

3. Remove plate from incubator. Eliminate air bubbles from wells if necessary. Measure absorbance (O.D.) at 492 nm using a plate reader.

4. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation.
   If incubation for 90 min, sample thiolase II activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 70 µl / (90 min × 0.4 cm × 18 × 20 µl) = **ΔO.D. × 5.4** (or **ΔO.D. x 4.05** for 120 min). Enzyme activity may be normalized by GAPDH enzyme activity (see cat#E-101) or protein concentration.

Additional information:

- The assay solution contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). Please refer to the product webpage or contact us for material SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.