BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Carnitine Palmitoyltransferase 1 (CPT1) Assay Kit (Cat #: E-163)

COMPONENTS: CPT1 Assay Solution- 5 ml (for 100 wells), store at -80°C **(shield solution from light during use)**
10x Palmitoyl-CoA- 0.5 ml, store at -80°C **(avoid multiple freeze-thaw cycles)**
10x Tissue Lysis Solution- 25 ml, store at 4ºC (dilute 10-fold with ice-cold dH_2O)
10x Lysis Buffer- 25 ml, store at 4ºC (dilute 10-fold with ice-cold dH_2O)

PRODUCT DESCRIPTION: The CPT1 activity assay is based on coenzyme A (CoA)-dependent reduction of iodonitrotetrazolium (INT) to formazan, which exhibits an absorption maximum at 492 nm ($\varepsilon = 18\ mM^{-1}cm^{-1}$) and allows for measurement of CPT1 activity in cell/tissue lysates. Reagents are stable for at least one year if stored and handled properly.

One-step preparation of cell/tissue lysates: for most cell/tissue type (except muscle and heart)

1. Prepare 1x Tissue Lysis Solution by diluting 10x Tissue Lysis Solution with dH_2O. Bring up at least ~10^5 PBS-washed cells in 50–100 µl ice-cold 1x Tissue Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Tissue Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Tissue Lysis Solution (~2 mg tissue in 0.2 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Harvest supernatant for CPT1 assay.
3. Use the BCA protein assay method to determine protein concentration. A suggested protein concentration range is 0.5 - 2 mg/ml. Normalization of sample protein concentration is recommended. Store samples at -80°C.

Two-step preparation of cell/tissue lysates: recommended for muscle & heart tissue

1. Cell/tissue samples should be frozen at -80°C prior to homogenization.
2. Bring up at least ~10^5 cells freeze-thawed cells in 50 – 100 µl ice-cold 1x Lysis Buffer by pipetting up and down. Freeze-thawed tissue is mechanically homogenized in ice-cold 1x Lysis Buffer (~2 mg tissue in 0.2 ml).
3. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Discard supernatant. Resuspend pellet in ~50 µl ice-cold 1x Tissue Lysis Solution by pipetting up and down thoroughly, making sure the membrane fraction is homogenized. Leave homogenate on ice for ~10 min with agitation. Repeat homogenization.
4. Follow steps 2-3 above.

Reagent thawing:

Keep thawed CPT1 Assay Solution and 10x Palmitoyl-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 CPT1 Assay Solution, e.g., 50 µl dH_2O + 500 µl CPT1 Assay Solution. Keep solution on ice shielded from light.

Reaction solution is prepared by mixing 1 part of 10x Palmitoyl-CoA (briefly vortex vial prior to pipetting) and 10 parts of CPT1 Assay Solution, e.g., 50 µl 10x Palmitoyl-CoA + 500 µl CPT1 Assay Solution. Gently mix 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. 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 30-120 min. *Do not use CO_2 incubator.*
3. Measure absorbance (O.D.) at 492 nm using a plate reader at 30, 60, 90, and 120 min.
4. Subtract control well reading from reaction well reading for each time point for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation. Select the time point with the greatest ΔO.D..

   If incubation for 30 min, sample CPT1 activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 70 µl / (30 min × 0.4 cm × 18 × 20 µl) = **ΔO.D. × 16.2**. For 60 min, **ΔO.D. x 8.1**. For 90 min, **ΔO.D. x 5.4**. For 120 min, **ΔO.D. x 4.05**.