BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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Lipase Assay Kit (Cat #: E-156)

COMPONENTS: 50x Lipase Reaction: 0.1 ml, store at room temperature (100 wells, **shield solution from light**)
50x Lipase Control: 0.1 ml, store at room temperature
Lipase Assay Buffer: 10 ml, store at room temperature
10x Cell Lysis Solution: 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The Lipase Assay Kit measures lipase activity using 4-nitrophenyl palmitate as substrate. The chromogenic substrate upon enzymatic hydrolysis yields 4-nitrophenol, which is measured by absorbance at 400-410 nm (molar extinction coefficient at 405 nm = 18 $mM^{-1}cm^{-1}$). The assay can be used for cell/tissue lysates, serum/plasma, and cultured cells/medium. Kit components are stable for at least one year if stored and handled properly.

Preparation of cell/tissue lysates and serum/plasma:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with dH_2O. Bring up ~10^5 washed cells in 50 –100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down. Leave homogenate on ice for 5 min with agitation. If homogenate is overly turbid, add more 1x Cell Lysis Solution and repeat homogenization. Tissue can be mechanically homogenized in ice-cold 1x Cell Lysis Solution (5 mg tissue in 0.5 ml).
2. Centrifuge homogenate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant (lysate) 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 0.1 – 3 mg/ml. *Normalization of sample protein concentration is recommended.*
4. Serum/plasma should be diluted 5-fold with ice-cold 1x Cell Lysis Solution prior to assay.

Preparation of reaction solution and control solution:

Control solution is prepared by mixing 1 part of 50x Lipase Control and 50 parts of Lipase Assay Buffer, e.g. 10 µl 50x Lipase Control mixed with 500 µl Lipase Assay Buffer. Vortex solution to mix.

Reaction solution is prepared by mixing 1 part of 50x Lipase Reaction and 50 parts of Lipase Assay Buffer, e.g. 10 µl 50x Lipase Reaction mixed with 500 µl Lipase Assay Buffer. Vortex solution to mix.

Enzyme assay:

1. Use a plain (uncoated) 96-wll plate for the assay. First add 20 µl of 1x Cell Lysis Solution to duplicate wells (for background control set and background reaction set).
2. Then add 20 µl of each sample to duplicate wells (for sample control set and sample reaction set).
3. Proceed to add 50 µl control solution to each well of the control set and 50 µl reaction solution to each well of the reaction set. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 30°C incubator for 60 min or 120 min. *Do not use CO_2 incubator*. The plate should be shielded from light during incubation.
4. Remove plate from incubator. Measure $O.D._{405\ nm}$ using a plate reader.
5. Subtract control well reading from reaction well reading to generate ΔO.D. for the background well and each sample. Then subtract $\Delta O.D._{Background}$ from $\Delta O.D._{Sample}$ to generate $\mathbf{\Delta\Delta O.D._{Sample}}$ for each sample.

   For 60-min incubation, lipase enzyme activity in IU/L = µmol/(L•min) = $\mathbf{\Delta\Delta O.D._{Sample}} \times 1000 \times 70\ \mu l$ / (60 min × 0.4 cm × 18 × 20 µl) = $\mathbf{\Delta\Delta O.D._{Sample}} \times \mathbf{8.1}$ (or **4.05** for 120-min incubation).
   Cell/tissue lipase activity may be presented as units/µg proteins or normalized by GAPDH enzyme activity.

Additional information:

- 50x Lipase Reaction and 50x Lipase Control contain isopropanol, which is flammable. 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.