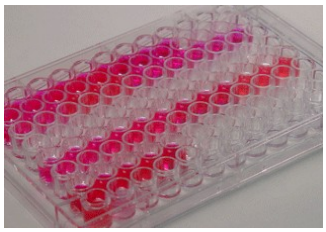
FT-CL0940

Long Wavelength Fluorescent Lipase Assay Kit

Allows fast and easy measurement of lipase activity *in vitro*, in cell preparations or *in vivo* using the long wavelength substrate resorufin oleate

Product Description



Name :	**Long Wavelength Fluorescent Lipase Assay Kit**
Catalog Number :	FP-CL0940 1 kit
Materials :	1 – **Reaction Buffer** 4 x 3 mL This buffer contains 100mM Glycine with 19mM sodium deoxycholate, pH 7,2. To prepare, add 3mL distilled water to reaction buffer vial and mix by inversion until all solid is dissolved. 2 - **Substrate Reagent** 1 x 2 mL 5mM Resorufin oleate in Dimethyl sulfoxide (DMSO). Dilute to émM for use in the assay protocol below. 3 – **Reference Standard** 1 x 250 µl 10mM Resorufin in Dimethyl sulfoxide (DMSO). Dilute to 2mM for use in the assay protocol below. 4 – **Cell Lysis Buffer** 1 x 10 mL 25mM Tris-Phosphate (pH 7,8) containing 10% glycerol, 1% Triton X-100, 1mg/ml BSA, 2mM EGTA and 2mM DTT. 5 – **DMSO** 1 x 5mL
Abs. / Em. :	$\lambda_{exc}\backslash\lambda_{em}$ (upon cleavage) = 571/587nm

Storage: The substrate reagent and reference standard included in this kit should be kept cold when not in use and stored at -20°C. Protect solutions of the substrate reagent and reference standard from light.

Introduction

Lipases are a family of enzymes that release fatty acids from triacylglycerols in a site specific manner. Most lipases have optimum activity for the primary ester groups of triglycerides, while some lipases remove fatty acyl groups from either the C-1 or C-3 acyl positions. The substrate is typically not a single molecule, but a nonaqueous phase of aggregated lipid. Lipase activity, ubiquitous among most cells, can be monitored using our new long wavelength fluorescent substrate, resorufin oleate (Product No. 2), contained in this kit. Upon cleavage, the fluorescent compound, resorufin (Abs 571nm, EM 587nm), is released and activity measurements are easily obtained either *in vitro*, in cell lysate preparations, or *in vivo*. The kit contains enough substrate for numerous assays and control experiments, and also contains reference standards and a detailed protocol for use. See the references below for more information and applications.

Directions for use

Enzyme Assay

It is recommended that a calibration curve be generated using concentrations of the Reference Standard in a range from 0 to the highest concentration of substrate reagent used for all assays.

In addition, purified enzyme assays should be performed using several enzyme concentrations in the approximate or estimated range of the enzyme concentration expected for the unknown sample. To normalize data, each enzyme reaction should be subtracted from a blank (no enzyme) sample.

1.) Cell Lysate samples are prepared as follows: Adherent or non-adherent cells are grown to 70-80% confluency using standard tissue culture conditions. The media is removed from the cell samples by suction or mild centrifugation, and the cells washed with sterile PBS (5 mL per 100 mm plate) and the PBS removed as above. Lysis Buffer (Product No. 4) is added to the cells (1 mL). Cells are incubated at 37°C for 30 mins. Lysate (1 mL) is collected by pipet and transferred to a small tube or vial. Lysate sample is placed in ice bath.
2.) Purified enzyme concentrations should be prepared fresh by diluting enzyme in reaction buffer (Product No. 1). The concentrations of purified enzyme should be in the range of 2.00 units/mL-200 units/mL.
3.) To a 96-well microtiterplate (clear, flat bottom) transfer samples containing cell lysate, purified enzyme, or cell suspension. Samples are added into wells on a microtiter plate in triplicate for each concentration and sample (50 µL/well). The concentrations of purified enzyme should be in the range of 2.00 units/mL-200 units/mL. Include in triplicate wells for blanks (50 µL reaction buffer/well for blanks of known enzyme concentrations, 50 µL of lysis buffer/well for blanks of cell lysate samples).
4.) Add reaction buffer (100 µL) to each well prepared in step (3) above. Allow a few minutes of incubation at room temperature to ensure a homogenous reaction mixture.
5.) Prepare 2mM reference standards by first diluting 10mM reference standard solution (60 µL) (Product No. 3) in ice-cold DMSO (240 µL) (Product No. 5) to make a 4X working solution. To wells to be used as reference standards, add 50uL of the prepared 2mM solution to 150µL reaction buffer. (see note (3) below). These wells will be the same final volume as the treatment and blank wells, and will have an equivalent concentration of reference standard to substrate reagent.
6.) Prepare a 2mM substrate reagent solution by diluting 5mM substrate reagent (400 uL) (Product No. 2) in ice-cold DMSO (600 µL) (Product No. 5) to make a 4X working solution. Cool this solution to 0°C by immersing in an ice-bath for about 10 min. (see Note (2) below). Add ice-cold 2mM substrate reagent (50 µL/well) to all wells, except those containing reference standard.
7.) Read fluorescence (Ex/Em = 571/585 nm) in a microtiter plate reader, using appropriate filters. Use the wells containing reference standard to optimize reading conditions. It is suggested that readings be taken beginning immediately after addition of the substrate reagent, and at ~1 min. intervals and for a period of at least 20 mins. Afterward.
8.) Average the readings of duplicate samples. Subtract fluorescence of blanks from that of each sample in order to normalize data. (see Note (2) below).
9.) Generate a calibration curve using purified enzyme samples by plotting normalized fluorescence vs. time (log-log).
10.) Using the calibration curve generated in step 9, determine the activity (concentration) of the enzyme in the original cell/tissue suspension.

Note (1.) *It is best to prepare the reaction buffer just prior to performing the assay. When stored cold (4˚C) after preparation, reaction buffer may be stable for several days. It is common for a precipitate to form in the reaction buffer after several days or exposure to high temperature.*

Note (2.) *Some nonspecific hydrolysis of the substrate reagent into the fluorescent resorufin product can occur spontaneously, particularly in high temperature or at pH values above 9.0. The reaction buffer included in this kit has been designed to minimize this nonspecific hydrolysis, though some hydrolysis may still occur. To further minimize this hydrolysis, cool the substrate reagent solution (A) to 0˚C (ice-bath) before addition to cold reaction buffer. Potential hydrolysis may necessitate the need for blank wells as recommended in the assay protocol.*

Note (3.) *Use of reference standard wells may be useful in calculating optimal gain with a microtiter plate reader.*

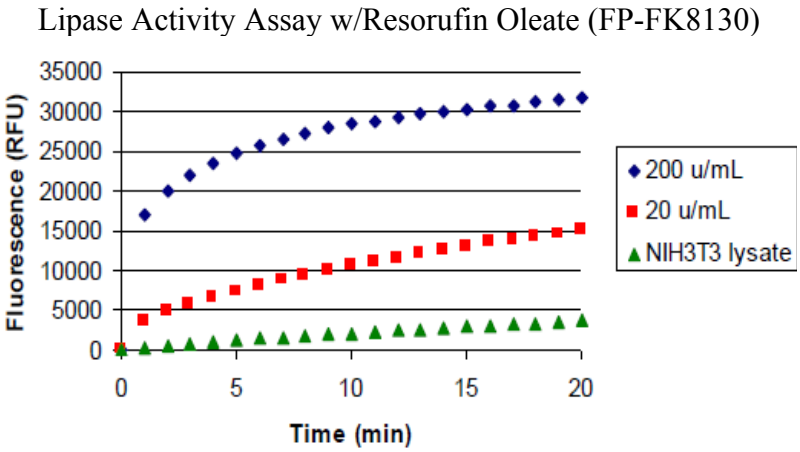


Figure 1: Two known concentrations (20.0 units/mL, 200 units/mL) of lipase (from *Candida ragosa*, SIGMA L-1754) were prepared using reaction buffer as diluent. Each known concentration, as well as cell lysate obtained from NIH3T3 mouse lymphoblast tumor cells (in lysis buffer), was added in triplicate to wells on a 96-well microtiter plate (50 µL/well). Three wells were prepared containing reaction buffer (50 µL) and no enzyme to be used as blanks for the known enzyme concentrations, and an additional three wells were prepared containing lysis buffer (50 µL) to be used as a blank for the cell lysate reaction mixture. Reaction buffer (100 µL) was added to each well, followed by 2mM Resorufin oleate (50 µL) in DMSO. The plate was immediately placed in a Perkin-Elmer 7000 Plus UV/FL/LUM Microtiter plate reader, and fluorescence was measured at EX/EM: 485/595 at one minute intervals for 20 minutes. The three wells for each sample/blank was averaged, and the blank values were subtracted from sample values at each time point.

References

1.) **Dousset, N.**, *et al*. "Use of a fluorescent radio labeled triacylglycerol as a substrate for lipoprotein lipase and hepatic triglyceride lipase." *Lipids* 23:605-608 (1988)
2.) **Liodakis, A**., *et al*. "Spectrofluorometric determination of lipase activity." *Biochem. International* 23(5): 825-834 (1991)
3.) **Main, L.A.**, *et al*. "Cholesterol ester transfer protein reaction between plasma lipoproteins." *J Biochem (Tokyo)* 124: 237-243 (1998).
4.) **Negre, A**., *et al*. "Hydrolysis of fluorescent pyrenetriacylglycerols by lipases from human stomach and gastric juice." *Biochim Biophys Acta* 963: 340-348. (1988)
5.) **Rosseneu, M.**, *et al*.. "Hydrolysis of very low-density lipoproteins labeled with a fluorescent triaglycerol: 1,3-dioleoyl-2-4(pyrenylbutanoyl)glycerol." *Eur J Biochem* 152: 195-198 (1985)

Technical and scientific information

Related / associated products and documents

See BioSciences Innovations catalogue and e-search tool.

- Resorufin Oleate FP-FK8130

Ordering information

Catalog size quantities and prices may be found at www.interchim.com/
Please inquire for higher quantities (availability, shipment conditions).
For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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