FT-BG8440

Lipase Assay Kit

Product Information

Name :	**Fluorescent Lipase Assay Kit**
Catalog Number :	FP-BG8440, 1 kit
Components :	Substrate Reagent. 120 µL of 30 mM DPG in n-butanol Reference Standard. 600 µL of 10 mM PDA in n-butanol Reaction Buffer 100 mM glycine buffer with 19 mM sodium deoxycholate, pH 9.5
Absorption / Emission :	$\lambda_{exc}\backslash\lambda_{em}$ (PDA excimer) = 340/470nm $\lambda_{exc}\backslash\lambda_{em}$ (PDA monomer) = 340/390nm

Storage: -20°C (J)

Introduction

Lipases are a family of hydrolytic 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 positions, others remove both C-1 and C-3 acyl groups. Only specific lipases will cleave the C-2 acyl group from triacylglycerols. The substrate is typically not a single molecule but a non-aqueous phase of aggregated lipid. Lipase activity, ubiquitous among most cells, can be monitored using the new fluorescent substrate kit that utilizes a fluorescent triglyceride 1,2dioleoyl-3-pyrenedecanoyl-*rac*-glycerol (DPG). Upon cleavage, the fluorescent fatty acid, pyrenedecanoic acid, is released and activity measurements are easily obtained either *in vitro* (in cell preparations) or *in vivo*. The fluorescent triglyceride substrate also will have applications to other research areas including micelle and membrane labeling, fluorescence energy transfer experiments, and membrane fluidity studies.

Directions for use

NOTE: The following information is given as a viable methodology for use of the Fluorescent Lipase Assay Kit. The user may determine their own best conditions for use dependent on the specific conditions present in their experiment.

●Components of the kit:
[A] **Substrate Reagent**: 120 mL of 30 mM 1,2-dioleoyl-3-pyrenedecanoyl-*rac*-glycerol (DPG) in n-butanol. (J)
[B] **Reference Standard**: 600 mL of 10 mM pyrenedecanoic acid (PDA) in n-butanol. (J)
[C] **Reaction Buffer**: This buffer contains 100 mM glycine buffer with 19 mM sodium deoxycholate, pH 9.5 . (K)

●Storage: Fluorescent reagents, fluorescently labeled lipids and fatty acids should be handled with care, kept cold (ice-bath) when not in use, and stored frozen (-20°C). Buffer can be stored at +4°C.

●Handling & Safety: In case of contact with skin or eyes, wash thoroughly with soap and cold water. Reagents should be stable for at least 6 months following purchase. High background fluorescence readings for blank samples will indicate decomposition.
These materials are intended for research purposes only. Use in drug or manufacturing processes is strictly prohibited.

Protocol 1 – Enzyme Assay(r)

Note: *It is recommended that a calibration curve be prepared using known concentrations of purified enzyme and a substrate emulsion* (such as a solution in reaction buffer containing 2.5:1 DPG (20%)/triolein: phosphatidylcholine)[1] in the approximate concentration range of the unknown analyte.ere is an increase in fluorescence emission of the monomer, which can also be monitored at 390nm (rem).

To normalize data and remove background fluorescence contribution, each enzyme reaction should be compared to a blank containing no substrate.

1.) The sample containing cell suspension is pipetted into wells of a microtiter plate in triplicate for each cell/tissue sample (50µL/well). Purified enzyme at a starting concentration of about 100units/ml should be serially diluted in triplicate to allow creation of a standard curve. Include three wells containing 50µl reaction buffer [C] for blanks.

2.) Add 100µL reaction buffer [C] to each well. Incubate for a few minutes to make sure the reaction system is homogeneous.

3.) Add 50µL 1 mM substrate reagent [A](dilute from provided solution using dH_2O) to each well.
Mix thoroughly (at 1 mM this kit contains enough buffer and substrate for 72 assays at 200µL total volume per well).

4.) Read fluorescence at 390 nm using an appropriate excitation filter at 340nm in a microtiter plate reader at 37°C (or temperature appropriate to your enzyme). Use the reference standard [B] for optimizing reader conditions.
Readings should be taken every 15 minutes for a total of 3.5 hours.

5.) Subtract fluorescence from the blank wells from each sample well.
Average the readings of triplicate samples.

6.) Generate a calibration curve using purified enzyme samples by plotting normalized fluorescence vs. time.
(This calibration step is only necessary for quantitative assay. For more details see note).

7.) Using the calibration curve from above, determine the activity (concentration) of lipase in samples by comparison, and extrapolate this data to determine concentration of the enzyme in the original cell/tissue suspension.

Note: Measurement of purified lipase activity using the kit substrate will provide a standard curve. The plot of activity for samples containing various concentrations of lipase enzyme and at various time points will generate a set of standard curves, one for each enzyme concentration. Only one such plot, however, is necessary to estimate enzyme concentration. This data will be used to estimate the relative amount of enzyme in test (cellular) samples with unknown lipase activity.

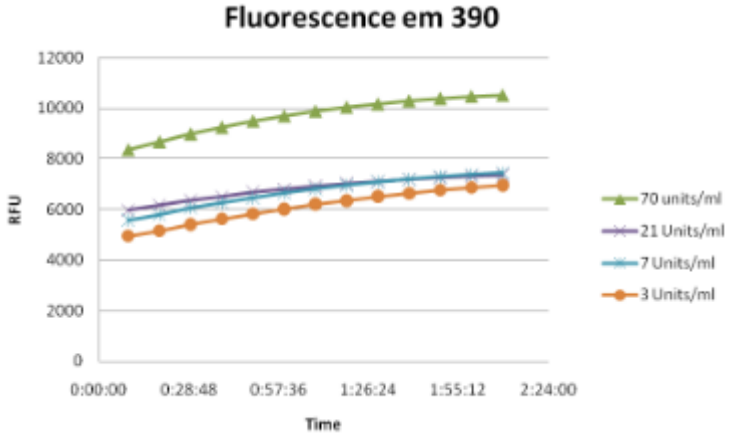

Figure 1. Purified Lipase was dissolved in Reaction Buffer at a starting concentration of 70 units/ml and then serially diluted 1 in 3 . To a 96 - well microtiterplate was added 50µl Lipase, 50µl 1mM Substrate and 100µl Reaction Buffer, in triplicate, (no lipase for blank controls) and fluorescence was measured at EX/EM: 340/ 390 n m using a BioTek Synergy MX Microtiterplate reader.

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Protocol 2 – to monitor decrease in excimer fluorescence (r)

Note: *It is recommended that a calibration curve be prepared using known concentrations of purified enzyme and a substrate emulsion* (such as a solution in reaction buffer containing 2.5:1 DPG (20%)/triolein: phosphatidylcholine)[1] in the approximate concentration range of the unknown analyte. Since the conversion of the fluorogenic substrate releases the fluorescent fatty acid monomer , pyrenedecanoic acid (PDA), the fluorescence emission of the substrate excimer decreases over time and is monitored at 470 nm using excitation at 340nm. Subsequently, there is an increase in fluorescence emission of the monomer, which can also be monitored at 390nm.

To normalize data and remove background fluorescence contribution, each enzyme reaction should be compared to a blank containing no substrate.

1.) The sample containing cell lysate, purified enzyme (325 - 650 units/mL), or cell suspension is pipetted into suitable individual cuvettes or wells of a microtiter plate with serial dilutions (4 dilutions for a total of 5 samples recommended) in duplicate for each concentration and for each cell/tissue sample (20 – 50µL/well). Include two cuvettes/wells for blanks (20 – 50µL/well reaction buffer).

2.) Add 100µL reaction buffer [C] to each cuvette. Incubate for a few minutes to make sure the reaction system is homogeneous.

3.) Add 50µL 2 mM substrate reagent [A](dilute from provided solution) to each well.
Mix thoroughly by manual agitation.
Note: Lower concentrations of the substrate reagent (0.5 - 2 mM) may be used if enzyme concentrations are sufficiently low or more assays are desired (at 2 mM this kit contains enough buffer and substrate for 36 assay s at 200µL total volume per well or 72 assays at 100µL total volume per well in a 96-well microtiter plate).

4.) Mix by inversion at a fixed temperature (normally 25ºC).
Note: It is possible that different enzymes require a higher temperature. Be sure to verify ideal temperature in the literature or product literature of the enzyme in question. Continue to mix for up to approximately 20 minutes after starting the reaction.

5.) Read excimer fluorescence at 470 nm using an appropriate excitation filter at 340nm in a spectrophotometer or microtiter plate reader. Use the reference standard [C] for optimizing spectrometer conditions.

6.) Read monomer fluorescence at 390nm, excitation also at 340nm. Excimer fluorescence should decrease o ver time as pyrenedecanoic acid is cleaved, subsequently increasing monomer fluorescence emission.

7.) Subtract fluorescence from the blank well(s) from each sample well.
Average the readings of duplicate samples.

8.) Generate a calibration curve using purified enzyme samples by plotting normalized fluorescence vs. time (log - log). (This calibration step is only necessary for quantitative assay. For more details see note).

9.) Using the calibration curve from above, determine the activity (concentration) of lipase in samples by comparison, and extrapolate this data to determine concentration of the enzyme in the original cell/tissue suspension.

10.) Additional information on these and alternate protocol conditions are given in the references listed below.

Note: Measurement of purified lipase activity using the kit substrate will provide a standard curve. The plot of activity for samples containing various concentrations of lipase enzyme and at various time points will generate a set of standard curves, one for each enzyme concentration. Only one such plot, however, is necessary to estimate enzyme concentration. Since the protocol doesn't specify the length (time) of the assay for samples, it will be necessary to plot the fluorescence versus time for the standard (enzyme) sample(s). This data will be used to estimate the relative amount of enzyme in test (cellular) samples with unknown lipase activity.

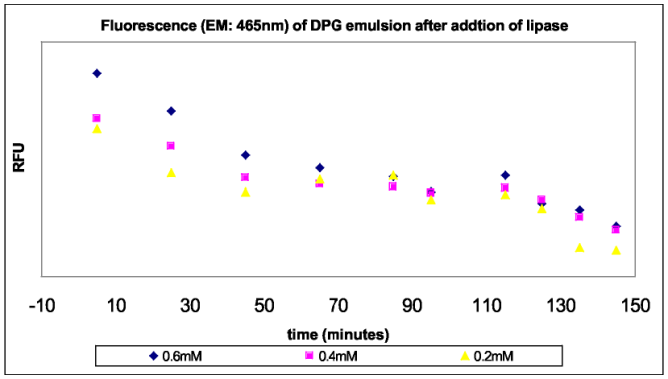

Figure 1. An emulsion of 1mM DPG:5mM Triolein:2.5mM egg Phosphatidylcholine was prepared by combining 100 µ L 30mM DPG Substrate reagent in BuOH, 150 µ L 100mM triolein in CH_2Cl_2 , and 250 µ L 30mM phosphatidylcholine in CH_2Cl_2 , drying in vacuo , and redissolving in 3mL Reaction Buffer. To a 96 - well microtiterplate was added DPG emulsion (to give emulsion concentrations shown in graph), ~10 units of pure lipase dissolved in H_2O, and Reaction Buffer, in duplicate, (no lipase for blank controls) and excimer fluorescence was measured at EX/EM: 340/470nm using a Perkin - Elmer HTS 7000 Plus UV/FL/LUM Microtiterplate reader.

Additional information on these and alternate protocol conditions are given in the references listed below.

REFERENCES

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Related products

- 1,2-dioleoyl-3-pyrenyldecanoyl-rac-glycerol (DPG), FP-M14031
- 1-pyrenedecanoic acid, FP-37853A
- Long Wavelength Fluorescent Lipase Assay Kit, FP-CL0940

Ordering information

Catalog size quantities and prices may be found at http://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