Lipase Activity Colorimetric Assay Kit II

(Catalog #BN00942; 100 assays; Store kit at -20°C)

I. Introduction:

Lipase is a subclass of the esterases that catalyze the hydrolysis of ester bonds in water-insoluble, lipid substrates. Lipases perform essential roles in the digestion, transport and processing of dietary lipids (e.g. triglycerides, fats, oils) in most, if not all, living organisms. In humans, pancreatic lipases are the key enzyme responsible for breaking down fats in the digestive system by converting triglycerides to monoglycerides and free fatty acids. During the damage of the pancreas, lipase levels can rise 5 to 10-fold within 24 to 48 hours. The kit provides a simple, sensitive, and reliable assay for rapid analysis of Lipase in samples. In the assay, lipases hydrolyze a specific substrate to generate a product which reacts with the DTNB probe to generate color (λ = 412 nm). The kit is also suitable for high throughput analyses.

II. Kit Contents:

Components	100 assays	Cap Code	Part Number
Lipase Assay Buffer	25ml	WM	BN00942-1
DTNB Probe (lyophilized)	1 vial	Red	BN00942-2
Lipase Substrate	0.5 ml	Blue	BN00942-3
TNB Standard (Lyophilized)	1 vial	Amber	BN00942-4
Lipase Positive Control (lyophilized)	1 vial	Purple	BN00942-5

III. Storage and Handling:

Store the kit at -20°C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge vials before opening. Read the entire protocol prior to performing the assay.

IV. Reagent Preparation:

DTNB Probe: Dissolve the DTNB Probe with 1.1 ml Lipase Assay Buffer. Store at -20°C. Use within two months.

Lipase Substrate: Ready to use. Store at -20°C. Use within two months.

TNB Standard: Dissolve with 0.5 ml of Assay Buffer to generate 5 mM TNB Standard. Stable for 2 months at -20°C

Lipase Positive Control: Dissolve the positive control with 100 µl Lipase Assay Buffer. Store at -20°C. Use within two months.

V. Lipase Assay Protocol:

1. **Standard Curve Preparation:**

   Add 0, 2, 4, 6, 8, 10 µl of TNB Standard into a series of wells. Adjust volume to 150 µl/well with Assay Buffer to generate 0, 10, 20, 30, 40, 50 nmol/well of TNB Standard.

2. **Sample Preparations:**

   Tissues or cells can be homogenized in 4 volumes of Assay Buffer and centrifuged (13,000 x g, 10 min) to remove insoluble material. Serum samples can be directly diluted in the Assay Buffer. Prepare test samples of up to 50 µl/well with Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure the readings are within the standard curve range. Mercaptans in samples will cause a high background. If the sample background is too high, the sample can be precipitated with 2 volumes of saturated ammonia sulfate. Then centrifuge, collect the precipitates and re-dissolve in the same volume of assay buffer to remove small molecule mercaptans.

3. **Positive Control (optional):**

   Add 5 µl of the reconstituted Lipase Positive Control into Positive Control well and adjust the volume to 50 µl/well with assay buffer.

4. **Reaction Mix:** Mix enough reagent for the number of assays to be performed. For each well, prepare a total 100 µl Reaction Mix:

   85 µl Assay Buffer
   10 µl DTNB Probe
   5 µl Lipase substrate

   Add 100 µl of the Reaction Mix to each well containing the Positive Controls and samples. Mix well. **(DO NOT ADD TO STANDARDS)**

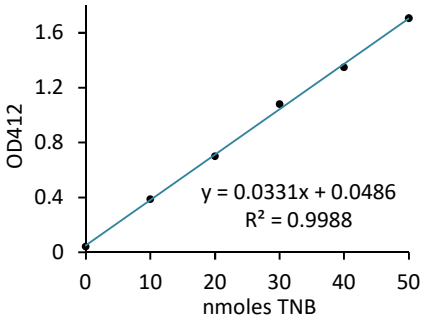
5. **Measurement:** Read OD 412 nm A_1 at T_1 after 3 min incubation time. Read A_2 OD 412 nm again at T_2 after incubating the reaction at 37°C for 60 - 90 min (or incubate longer time if the Lipase activity is low), protect from light. The OD of color generated upon formation of TNB is ΔA 412 nm = $A_2 - A_1$. It is recommended to read kinetically to choose the A_1 and A_2values which are in the linear range of the Standard Curve.

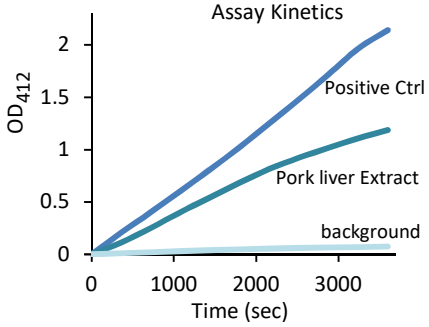
6. **Calculation:** Subtract 0 Standard from all standard readings. Plot the Standard Curve. Apply the ΔA 412 nm of samples to the standard curve to get B nmol of TNB generated in the sample reaction between T_1 and T_2. Lipase activity in samples can then be calculated:

   Lipase Activity = $\frac{B}{(T2-T1)\times V}$ **x Sample Dilution Factor = nmol/min/ml = mU/ml**

   Where: **B** is the TNB amount calculated from the Standard Curve (in nmol).
   T_1 is the time of the first reading (A_1) (in min).
   T_2 is the time of the second reading (A_2) (in min).
   V is the pretreated sample volume added into the reaction well (in ml).

   Unit Definition: One unit lipase is defined as the amount of lipase which hydrolyzes the substrate and generates 1.0 µmol of TNB per minute at 37°C.





FOR RESEARCH USE ONLY! Not to be used on humans.

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures until use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		

GENERAL TROUBLESHOOTING GUIDE: