Triglyceride Quantification Kit

(Catalog #K622-100; 100 assays; Store at -20°C)

I. Introduction:

Triglycerides (TG) are the main constituent of vegetable oil, animal fat, LDL and VLDL, and play an important role as transporters of fatty acids as well as serving as an energy source. TG are broken down into fatty acids and glycerol, after which both can serve as substrates for energy producing and metabolic pathways. High blood levels of TG are implicated in atherosclerosis, heart disease and stroke as well as in pancreatitis. The Triglyceride Quantification Kit provides a sensitive, easy assay to measure TG concentration in a variety of samples. In the assay, TG are converted to free fatty acids and glycerol. The glycerol is then oxidized to generate a product which reacts with the probe to generate color (spectrophotometry at λ = 570 nm) and fluorescence (Ex/Em = 535/587 nm). The kit can detect 2 pmol-10nmol (or 2-10000µM range) of triglyceride in various samples. The kit also detects monoglycerides and diglycerides.

II. Kit Contents:

Components	K622-100	Cap Code	Part Number
Triglyceride Assay Buffer	25 ml	WM	K622-100-1
TriglycerideProbe (in DMSO, anhydrous)	200 µl	Red	K622-100-2A
Lipase	1 vial	Blue	K622-100-4
TriglycerideEnzyme Mix (lyophilized)	1 vial	Green	K622-100-5
Triglyceride Standard (1 mM)	0.3 ml	Yellow	K622-100-6

III. Storage and Handling:

Store kit at –20°C, protect from light. Warm Triglyceride Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

IV. Reagent Preparation:

TRIGLYCERIDE STANDARD: Frozen storage may cause the triglyceride standard to separate from the aqueous phase. To re-dissolve, keep the cap tightly closed, place in a hot water bath (~80-100°C) for 1 min or until the standard looks cloudy, vortex for 30 sec, the standard should become clear. Repeat the heat and vortex one more time. The triglyceride standard is now completely in solution, and ready to use.

Triglyceride Probe: Ready to use as supplied. Warm to by placing in a 37 °C bath for 1-5 min to thaw the DMSO solution before use. (**Note: DMSO tends to be a solid after -20 °C storage, even when left at room temperature- so need to melt for a few min at 37 °C)**. Store at –20°C, protect from light. Use within two months.

Triglyceride Enzyme Mix: Dissolve in 220 µl Triglyceride Assay Buffer. Aliquot and store at –20 °C. Use within two months.

Lipase: Dissolve in 220 µl Triglyceride Assay Buffer. Aliquot and store at -20 °C. Use within two months.

V. Triglyceride Assay Protocol:

1. Standard Curve Preparation:

For the colorimetric assay, Dilute 40 µl of the 1 mM Triglyceride into 160 µl Assay Buffer, mix to generate 0.2 mM Triglyceride standard. Add 0, 10, 20, 30, 40, 50 µl of the 0.2 mM Triglyceride Standard into a series of wells. Adjust volume to 50 µl/well with Triglyceride Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Triglyceride Standard.

For the fluorometric assay, dilute the Triglyceride Standard to 0.01- 0.1 mM with the Triglyceride Assay Buffer (Detection sensitivity is 10-100 fold higher for a fluorometric than a colorimetric assay). Follow the procedure as the colorimetric assay.

2. Sample Preparation:* Prepare test samples to a final volume of 50 µl/well with Triglyceride Assay Buffer in a 96-well plate. We suggest testing several dilutions of your sample to make sure the readings are within the standard curve range.

***Note:** Serum contains 0.1-6 mM triglyceride, which can be tested directly.For tissue (~100 mg),cells (~10 million) or other non-aqueous samples, homogenize in 1 ml solution containing 5 % NP-40 in water, slowly heat the samples to 80-100 °C in a water bath for 2-5 min or until the NP-40 becomes cloudy, then cool down to room temperature. Repeat the heating one more time to solubilize all triglyceride. Centrifuge for 2 min (top speed using a microcentrifuge) to remove any insoluble material. Dilute 10 fold with dH_2O before the assay.

3. Lipase: Add 2µl of lipase to each standard and sample well. Mix and incubate 20 min at room temperature to convert triglyceride to glycerol and fatty acid.
Note: If samples contain glycerol, do a sample background control, omit the lipase to determine glycerol background only, not triglyceride.

4. Triglyceride Reaction Mix: Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix:

46 µl Triglyceride Assay Buffer
2 µl Triglyceride Probe**
2µl Triglyceride Enzyme Mix

****Note:** For the fluorometric assay, use 0.4 µl/well of the Probe to decrease the background readings, therefore increase detection sensitivity.

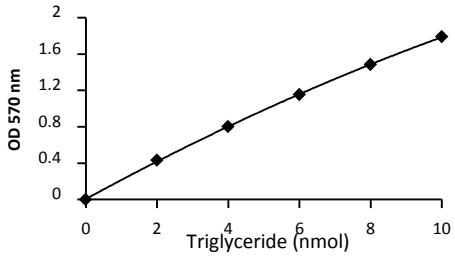
5. Mix well. Add 50 µl of the Reaction Mix to each well containing the Triglyceride Standard, test samples and controls. Mix well. Incubate at room temperature for 30-60 min- 60 min gives slightly better result, protect from light.

6. Measure OD 570 nm for colorimetric assay or Ex/Em = 535/590 nm for fluorometric assay in a microtiter plate reader. The reaction is stable for at least two hr.

7. Calculations: Correct background by subtracting the value derived from the 0 triglyceride standard from all sample readings. Plot the standard curve. Apply sample readings to the standard curve. Triglyceride concentration can then be calculated:

C = Ts / Sv nmol/µl or µmol/ml or mM

Where: **Ts** is triglyceride amount from standard curve (nmol).
Sv is the sample volume (before dilution) added in sample wells(µl).



RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents	Glucose and Sucrose Assay Kit
Cholesterol, LDL/HDL Assay Kits	Lactate, Pyruvate Assay Kit
Glutathione Assay Kits	Ethanol and Uric Acid Assay Kits
NAD/NADH and NADP/NADPH Assay Kit	

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

GENERAL TROUBLESHOOTING GUIDE:

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
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• 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, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till 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.		