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ab178780 Picoprobe Triglyceride Quantification Assay Kit (Fluorometric)

For the rapid, sensitive and accurate measurement of triglycerides in various biological samples.

This product is for research use only and is not intended for diagnostic use.

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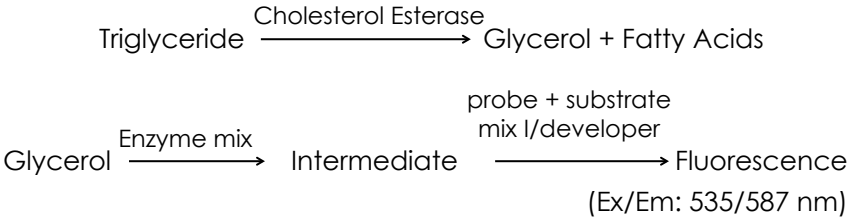
1. Overview

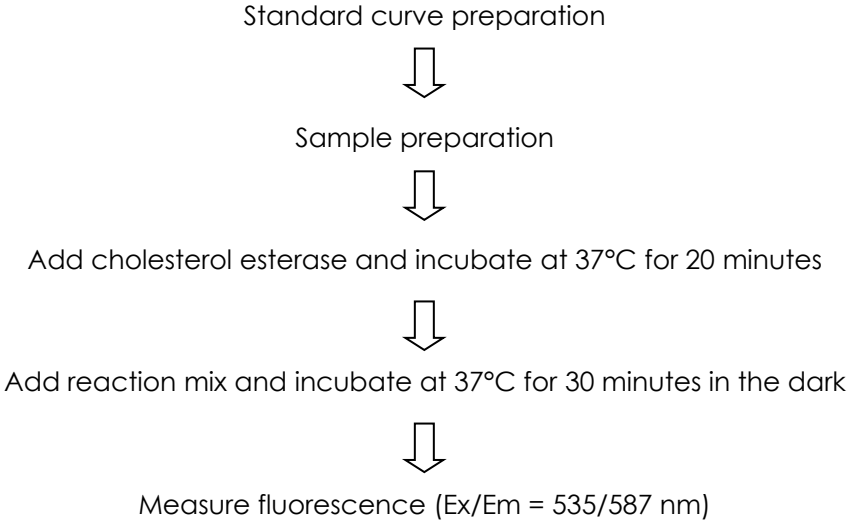
PicoProbe Triglyceride Quantification Assay Kit (Fluorometric) (ab178780) is a sensitive and easy-to-use kit, suitable for measuring triglyceride levels in samples which contain reducing agents that may interfere with oxidase-based assays. In this assay, triglycerides are hydrolyzed to free fatty acids and glycerol. The glycerol reacts with the Triglyceride Enzyme Mix to form an intermediate product, which in turn reacts with the PicoProbe I probe and Substrate Mix I/developer to generate fluorescence that can be detected at Ex/Em = 535/587 nm. The generated fluorescence is directly proportional to the amount of triglycerides present in the sample.

This high-throughput (HTP) adaptable assay kit is simple, sensitive and easy to use. It detects less than 0.4 µM triglycerides present in cell or tissue lysates or saliva.



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. Triglycerides 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 triglycerides are implicated in atherosclerosis, heart disease and stroke as well as in pancreatitis.

2. Protocol Summary



3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition (Before prep)	Storage Condition (After prep)
Assay Buffer 5	25 mL	-20°C	-20°C
PicoProbe I	0.4 mL	-20°C	-20°C
Cholesterol Esterase	1 vial	-20°C	-20°C
Triglyceride Enzyme Mix	1 vial	-20°C	-20°C
Substrate Mix I	1 vial	-20°C	-20°C
Triglyceride Standard	300 µL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 5 was previously labelled as Assay Buffer V and Triglyceride Assay Buffer. The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom, white wells
- Dounce homogenizer (if using tissue)

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer 5:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 PicoProbe I:

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored at -20°C, even when let at room temperature, so it needs to melt for few minutes at 37°C.

Aliquot probe so that you have enough volume to perform the desired number of assays. Store at - 20°C protected from light and moisture. Once the probe is thawed, use within two months.

9.3 Cholesterol Esterase:

Reconstitute the Cholesterol Esterase in 220 µL Assay Buffer 5. Keep on ice while in use. Aliquot Cholesterol Esterase so that you have enough volume to perform the desired number of assays. Store at - 20°C. Use within two months.

9.4 Triglyceride Enzyme Mix:

Reconstitute Enzyme Mix in 220 µL Assay Buffer 5. Keep on ice while in use. Aliquot Enzyme Mix so that you have enough volume to perform the desired number of assays. Store aliquots at - 20°C. Use within two months.

9.5 Substrate Mix I:

Reconstitute Substrate Mix I/Developer in 220 µL ddH_2O. Keep on ice while in use. Aliquot Substrate Mix I/Developer so that you have enough volume to perform the desired number of assays. Store aliquots at - 20°C. Use within two months.

9.6 Triglyceride Standard:

Frozen storage may cause the triglyceride standard to separate from the aqueous phase. To re-dissolve, keep the cap tightly closed and place in a hot water bath (~80-100°C) for 1 minute or until the standard looks cloudy, and then vortex for 30 seconds, 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.

Aliquot standard so that you have enough volume to perform the desired number of assays. Store aliquots at - 20°C.

Δ Note: each aliquot of standard should be boiled as described above before every use.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 20 µM (20 pmol/µL) Triglyceride Standard by adding 20 µL of the 1 mM Triglyceride Standard into 980 µL of ddH_2O and mixing well by pipetting up and down.

10.2 Prepare a standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	TG Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount TG in well (pmol/well)
1	0	150	50	0
2	6	144	50	40
3	12	138	50	80
4	18	132	50	120
5	24	126	50	160
6	30	120	50	200

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10^6 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend cells in 100 µL ice cold Assay Buffer 5.
11.1.4 Homogenize cells quickly by pipetting up and down a few times.
11.1.5 Incubate sample on ice for 10 minutes.
11.1.6 Centrifuge for 5 minutes at top speed using a cold microcentrifuge to remove any insoluble material.
11.1.7 Collect supernatant
11.1.8 Transfer to a new tube.
11.1.9 Keep on ice.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg tissue).
11.2.2 Wash cells with cold PBS.
11.2.3 Resuspend tissue in 100 µL ice cold Assay Buffer 5.
11.2.4 tissue with a Dounce homogenizer or pestle sitting on ice, with 10 – 15 passes.
11.2.5 Incubate sample on ice for 10 minutes.
11.2.6 Centrifuge for 5 minutes at top speed using a cold microcentrifuge to remove any insoluble material.
11.2.7 Collect supernatant and transfer to a new tube.
11.2.8 Keep on ice.

11.3 Saliva:

11.3.1 Centrifuge saliva for 2 minutes at 5,000 rpm using a cold microcentrifuge to remove any insoluble material.
11.3.2 Collect supernatant and transfer to a new tube.
11.3.3 Keep on ice.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ Note: Glycerol and/or high levels of NADH present in cell or tissue extracts can generate background in this assay. If you suspect your samples contain glycerol or high levels of NADH, set up Sample Background Controls.

12.1 Reaction wells set up:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 5).
- Sample Background Control wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 5).

12.2 Assay procedure:

12.2.1 Add 2 µL of cholesterol esterase to Standard, Sample and Sample Background Control wells. Mix and incubate reaction at 37°C for 20 minutes.

12.2.2 Prepare 50 µL of Reaction Mix and mix enough reagents for the number of assays to be performed.

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer 5	45	47
PicoProbe I	1	1
Triglyceride Enzyme Mix	2	0
Substrate Mix I	2	2

12.2.3 Add 50 µL of the Reaction Mix into each standard and sample wells and 50 µL of the Background Reaction Mix to sample background control wells. Mix well.

12.3 Plate measurement:

12.3.1 Incubate plate (white wells, clear bottom) at 37°C for 30 min protected from light.

12.3.2 Measure output on a microplate reader at Ex/Em = 535/587 nm.

13. Calculations

– Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

13.1 Subtract the mean fluorescence value of the blank (Standard #1) from all readings. This is the corrected fluorescence.

13.2 If sample background control is significant, subtract the sample background control from sample reading.

13.3 Average the duplicate reading for each standard and sample.

13.4 Plot the corrected fluorescence values for each standard as a function of the final concentration of triglyceride.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Concentration of triglyceride (pmol/mL) in the test samples is calculated as:

$$\text{TG concentration} = \left(\frac{Ts}{Sv}\right) * D$$

Where:

Ts = TG amount in the sample well calculated from standard curve (pmol or nM).

Sv = sample volume added in the sample wells (µL).

D = sample dilution factor.

Triglyceride molecular weight = 885.4 g/mol

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

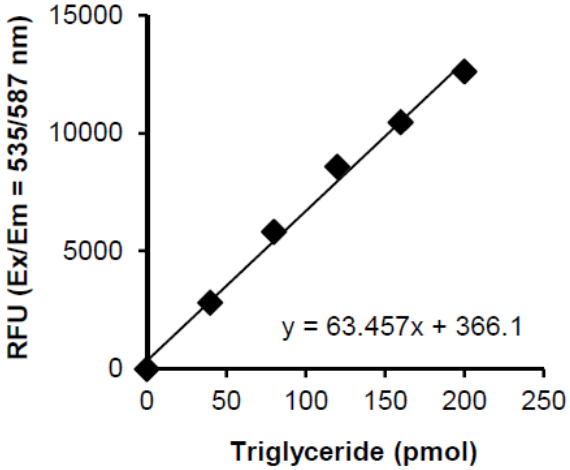


Figure 1. Typical standard calibration curve.

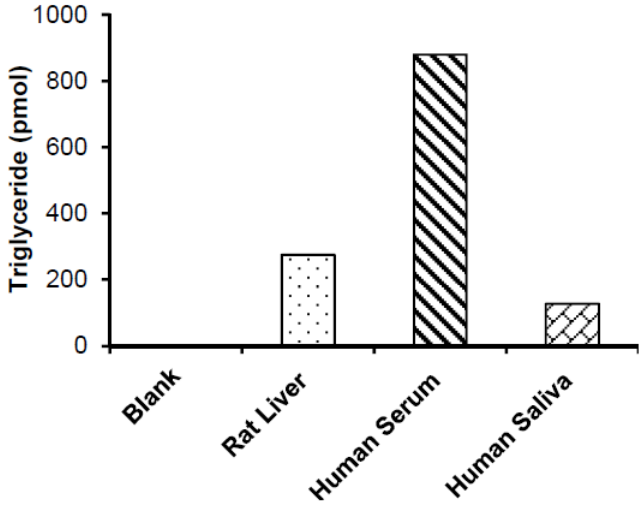


Figure 2. Measurement of triglyceride levels in rat liver (15 µg); human serum (1 µL) and human saliva (10 µL).

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Solubilize TG standard, thaw PicoProbe I probe and prepare Cholesterol Esterase, enzyme mix and substrate mix I/developer (aliquot if necessary); get equipment ready.
- Prepare TG standard dilution [40 - 200 pmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL), samples (50 µL) and background sample control wells (50 µL).
- Add 2 µL of Cholesterol Esterase to all wells. Incubate at 37°C for 20 minutes.
- Prepare a master mix for Reaction Mix and (if appropriate) a master mix for Background Reaction Mix:

Component	Reaction Mix (µL)	Bckg Reaction Mix (µL)
Assay Buffer 5	45	47
PicoProbe I	1	1
TG Enzyme Mix	2	0
Substrate Mix I/TG Developer	2	2

- Add 50 µL Reaction Mix to standard and sample wells.
- Add 50 µL Background Reaction Mix to Sample Background control wells.
- Incubate plate at 37°C for 30 minutes protected from light.
- Measure plate at Ex/Em= 535/587 nm.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. Interferences

These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure:

- Glycerol: can interfere with the cholesterol esterase activity and generate background.
- NADH: can generate high background.
- BSA: ≥ 1% can interfere with this assay.
- PMSF

18. FAQs

Q. What is the difference between this product and Triglyceride Quantification Kit (Colorimetric/Fluorometric) (ab65336)?

A. The PicoProbe present in this kit makes the assay more sensitive than ab65336. This product detects < 0.4 µM triglycerides, while ab65336 can detect 2 µM – 10 mM triglycerides.

Q. Is Deproteinizing recommended?

A. Deproteinization is not required for this assay.

Q. Will this assay detect di- and mono-acyl glycerols?

A. The Cholesterol Esterase enzyme that hydrolyzes triglycerides is specific for only tri-acylglycerol and therefore mono- and di-glycerides will not be recognized in this assay.

Q. Can tissue be stored in PBS-glycerol before the sample preparation step?

A. Glycerol creates background and hence tissue/cell storage in glycerol must be avoided. If essential for the sample, then glycerol needs to be washed off before the homogenization step.

Q. We generally use black plates for fluorometric assays, but you recommend white plates for this assay. Could you explain why?

A. Black plates are typically recommended for fluorescent assays; but for products containing Picoprobe reagent, white plates help to reflect more light thus increasing the sensitivity of the assay.

Q. The values of the standard curve are in the low hundreds. Why?

It is important to warm up the probe to >20C and the assay buffer to RT to ensure that the reaction proceeds according to the normal time of incubation. Incompletely thawed or cold reagents can lead to a slow reaction and low values.

Q. What can cause background in this assay?

Glycerol and NADH are the primary sources of background in this assay. Subtraction of background will help correct for this contribution. BSA (1% or higher) and PMSF might also interfere with

the assay.

Q. How are samples normalized against protein concentration?

A protein quantitation assay can be used with the supernatants from cell/tissue lysates or with any other liquid sample in the assay buffer.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)