ab102513
Adipogenesis Detection Assay Kit (Colorimetric/Fluorometric)

Instructions for Use

For rapid, sensitive and accurate measurement of adipogenesis in various samples.

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

Version 4 Last Updated 2 July 2019

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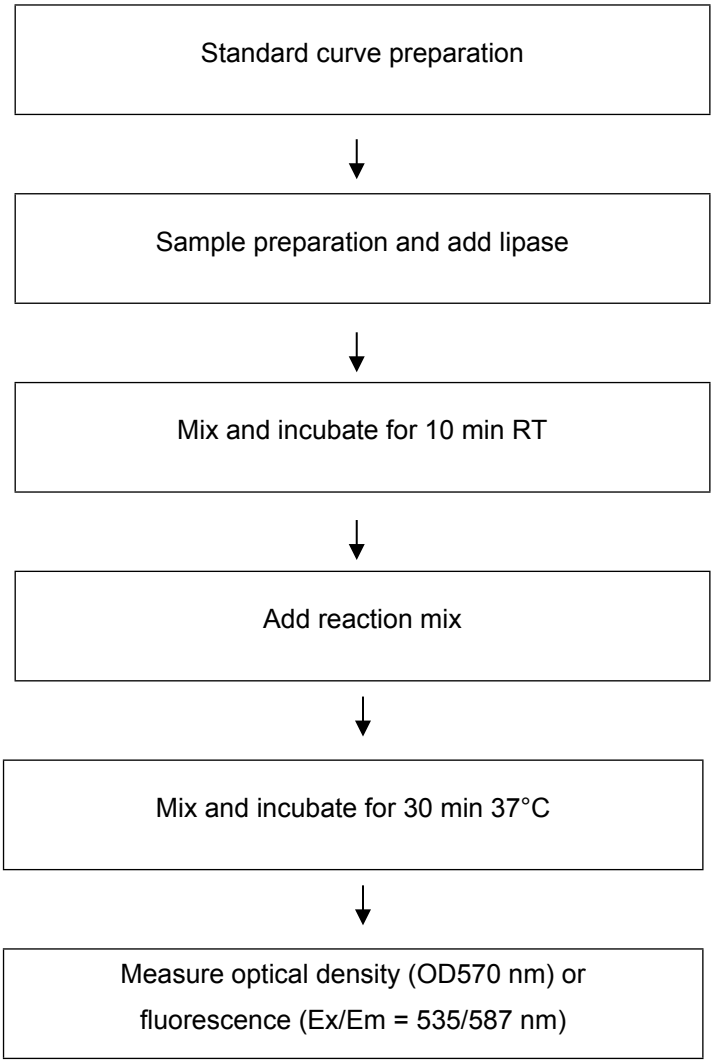
1. BACKGROUND

Adipogenesis Assay Kit (Colorimetric/Fluorometric) (ab102513) quantifies triglyceride accumulation in cells and tissues. In the assay, triglycerides are efficiently solubilized then hydrolyzed to glycerol which is subsequently oxidized to convert the probe to generate color (ODmax = 570 nm) and fluorescence (Ex/Em = 535/587 nm). The kit can detect 0.2 – 10 nmol of triglyceride in <1,000 differentiated 3T3-L1 cells. The high detection sensitivity and the convenient microplate assay format make the kit a convenient tool for studying the effect of adipogenesis inducers or to screen inhibitor compounds.

Adipogenesis is the process of differentiation of different cell types into adipocytes, the primary fat storage cell type. The accumulation of adipocytes is the basis for obesity, a significant risk factor in many diseases, including diabetes, atherosclerosis, cancer and cardiovascular disease, etc. Adipocytes accumulate triglycerides in the form of lipid droplets, which can be measured.

2. ASSAY 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. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC 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 section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Adipogenesis Assay Buffer	25 mL	-20°C	-20°C
Lipid Extraction Solution	10 mL	-20°C	-20°C
Adipogenesis Probe (in DMSO)	200 µL	-20°C	-20°C
Lipase (lyophilized)	1 vial	-20°C	-20°C
Adipogenesis Enzyme Mix (lyophilized)	1 vial	-20°C	-20°C
Triglyceride standard (1 mM)	300 µL	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader – equipped with filter for OD 570 nm or Ex/Em = 535/587 nm (respectively
- 96 well plate: clear plates for colorimetric assay; black plates (clear bottoms) for fluorometric assay
- Heat block or water bath
- Adhesive cover (if using cells)
- Dounce homogenizer or pestle (if using tissue)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- 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.

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.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- 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.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **Adipogenesis Assay Buffer:**

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

9.2 **Lipid Extraction Solution:**

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

9.3 **Triglyceride Standard (1 mM):**

Frozen storage may cause the standard to separate from the aqueous phase. To redissolve, place the tightly closed tube in a hot water bath (~80-100°C) for 1 minute or until liquid is cloudy. Vortex for 30 seconds. The solution should become clear. Repeat the heat/vortex procedure one more time. The standard is now in solution and ready to use. Store at -20°C.

9.4 **Adipogenesis Probe:**

Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left 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. Keep on ice while in use.

9.5 **Adipogenesis Enzyme Mix:**

Reconstitute with 220 µL Assay Buffer. Aliquot enzyme mix so that you have enough volume to perform the desired number of assays. Store at -20°C. Once thawed, use within two months. Keep on ice while in use.

9.6 **Lipase:**

Reconstitute with 220 µL Assay Buffer. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store at -20°C. Once thawed, use within two months. Keep on ice while in use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

10.1 **For the colorimetric assay:**

10.1.1 Prepare 400 µL of 0.2 mM Triglyceride standard, by diluting 80 µL of the provided 1 mM Triglyceride standard with 320 µL Assay Buffer.

10.1.2 Using 0.2mM Triglyceride standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of 0.2 mM Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End conc Triglyceride in well
1	0	125	50	0 nmol/well
2	25	100	50	2 nmol/well
3	50	75	50	4 nmol/well
4	75	50	50	6 nmol/well
5	100	25	50	8 nmol/well
6	125	0	50	10 nmol/well

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

10.2 **For the flurometric assay:**

10.2.1 Prepare 50 µL of 0.2 mM Triglyceride standard, by diluting 10 µL of the provided Triglyceride standard with 40 µL Assay Buffer.

10.2.2 Prepare 400 µL of 0.02 mM standard by diluting 40 µL of 0.2mM Standard with 360 µL Assay Buffer.

10.2.3 Using 0.02 mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of 0.02 mM Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End conc Triglyceride in well
1	0	150	50	0 nmol/well
2	30	120	50	0.2 nmol/well
3	60	90	50	0.4 nmol/well
4	90	60	50	0.6 nmol/well
5	120	30	50	0.8 nmol/well
6	150	0	50	1 nmol/well

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

NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

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 complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Adipocyte precursor cells (3T3 or human preadipocytes):

11.1.1 Culture cells in a 96-well plate (initial recommendation = 1,000 – 10,000 cells). Treat cells with desired reagents and methods.

11.1.2 When cells are ready for triglyceride testing, remove medium completely from wells and wash once with PBS.

11.1.3 Add 100 µL Lipid Extraction Solution to each well containing cells and seal plate with an adhesive cover to prevent evaporation.

11.1.4 Place entire plate in plate heater or heating block at 90 – 100°C for 30 minutes. The solution in the wells will become cloudy when heated. Cool the plate to room temperature.

11.1.5 Mix solution by shaking plate for 1 minute: triglycerides are now completely dissolved in the Lipid Extraction Buffer.

NOTE: *If oil droplets are still observed, reduce the number of cells used per assay. If only a few wells are to be tested, not the whole plate, pipette the Extraction Solution up and down 3-4 times in the culture wells, rinsing the well bottom to fully*

suspend the lipid droplets in the Lipid Extraction Solution. Complete mixing can be confirmed under microscope under 4-10X power, droplets will be seen uniformly dispersed through the depth of the Extraction Solution, not associated with the well bottom.

11.2 **Tissue samples:**

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Resuspend tissue in 100 µL of Lipid Extraction Solution.

11.2.4 Homogenize tissue with a Dounce homogenizer on ice, with 10 – 15 passes.

11.2.5 Incubate on ice 10 – 30 minutes.

11.2.6 Centrifuge 5 minutes at top speed in a cold microcentrifuge to remove debris/insoluble material. Transfer supernatant to a new tube.

11.2.7 Add extracted solution to wells in a 96-well plate and seal the plate with an adhesive cover to prevent evaporation.

11.2.8 Place entire plate in plate heater or heating block at 90 – 100°C for 30 minutes. The solution in the wells will become cloudy when heated. Cool the plate to room temperature.

11.2.9 Mix solution by shaking plate for 1 minute: triglycerides are now completely dissolved in the Lipid Extraction Buffer.

NOTE: *Protein concentration of the lipid extracts can be tested and used as an internal control to normalize the lipid concentration in the sample. We suggest using a detergent insensitive protein assay such as BCA method.*

For unknown samples, we suggest testing different doses of your sample to make sure the readings are within the standard curve range.

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**
- **3T3 cells can accumulate exceedingly large amounts of triglyceride: fully differentiated cells can contain up to 100X more triglyceride than un-induced cells. The amount of the lipid extract used for the assay will depend on the cell type, treatment and cell differentiation stage.**

12.1 **Set up Reaction wells:**

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

12.2 Add 2 µL Lipase to all sample and standard wells. (This converts triglyceride to glycerol and fatty acid.)

12.3 Mix and incubate for 10 minutes at room temperature**.**

12.4 **Reaction Mix:**

Prepare 50 µL of Reaction Mix for each reaction

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)
Adipogenesis Assay Buffer	**46**	**47.8**
Probe	**2**	**0.2**
Enzyme Mix	**2**	**2**

**For fluorometric readings, using 0.2 µL/well of the probe decreases the background readings, therefore increasing detection sensitivity.*

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:
X µL component x (Number samples + Standards +1).

12.5 Add 50 µL of reaction mix to each well. Mix well.

12.6 Incubate at 37°C for 30 minutes protected from light.

12.7 Measure output on a microplate reader.

- Colorimetric assay: measure OD570 nm.
- Fluorometric assay: measure 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 multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of triglyceride.

13.4 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).

13.5 Extrapolate sample readings from the standard curve plotted using the following equation:

$$A = \left(\frac{Corrected\ absorbance - y\text{-}intercept}{Slope}\right)$$

13.6 Concentration of triglyceride (nmol/µL or µmol/mL or mM) in the test samples is calculated as:

$$TG = \left(\frac{A}{B}\right) * D$$

Where:

A = Amount of triglyceride in the sample well (conc).

B = Sample volume added into the reaction well (µL).

D = Sample dilution factor

If desired, the sample triglyceride can be normalized to nmol per 10^6 cells, or per mg protein or tissue.

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14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

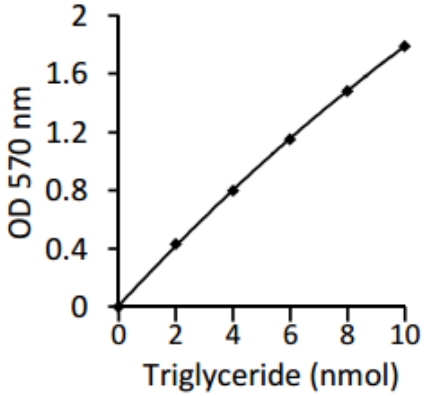

Figure 1. Typical triglyceride standard calibration curve using colorimetric reading.

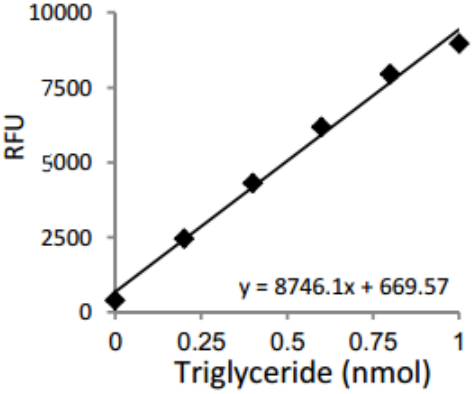

Figure 2. Typical triglyceride standard calibration curve using fluorometric reading.

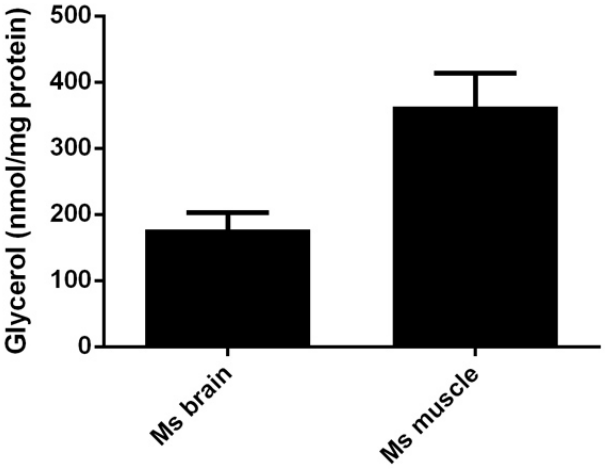

Figure 4: Glycerol measured fluorometrically in mouse tissue lysates showing quantity (nmol) per mg protein of tested sample.

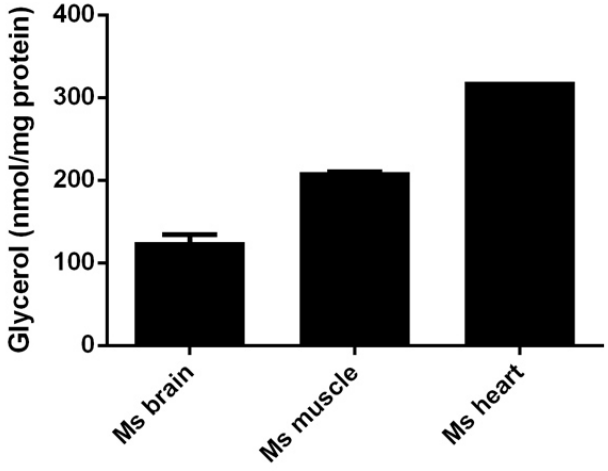

Figure 5: Glycerol measured colorimetrically in mouse tissue lysates showing quantity (nmol) per mg protein of tested sample.

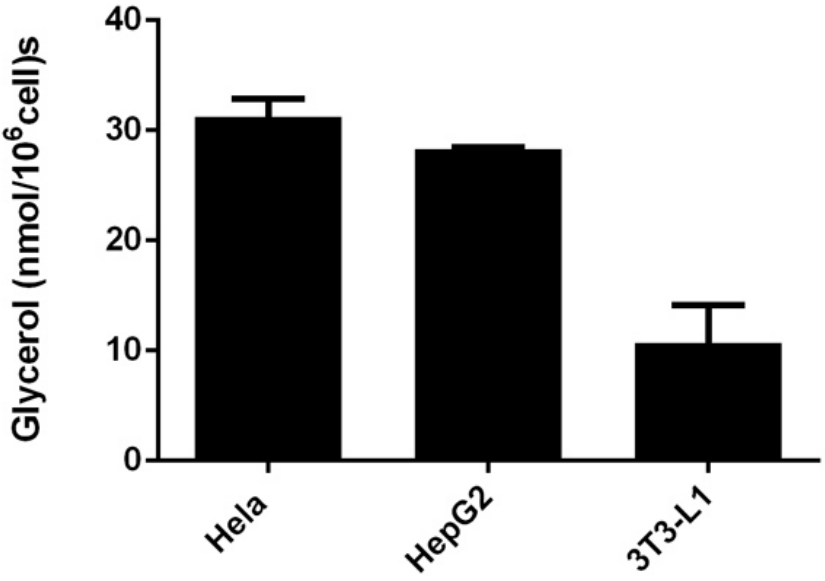

Figure 6: Glycerol measured colorimetrically in cell lysates showing quantity (nmol) per 1 mln of tested cells.

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, probe, lipase and prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL) and samples (50 µL) wells.
- Add 2 µL Lipase.
- Mix and incubate at RT for 10 mins.
- Prepare Reaction Mix (Number samples + standards + 1).

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)
Adipogenesis Assay Buffer	**46**	**47.8**
Probe	**2**	**0.2**
Enzyme Mix	**2**	**2**

- Add 50 µL of Reaction Mix to wells.
- Incubate plate at 37°C 30 minutes protected from light.
- Measure plate at OD570 nm for colorimetric assay or Ex/Em= 535/587 nm for fluorometric assay.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	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	Cause	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 on 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. FAQ

Can protein content be used as an internal control for this assay?

Yes, a detergent-compatible BCA assay (for example, BCA Protein Quantification Kit (ab102536)) can be used for protein quantitation to normalize sample amount.

Will the Assay buffer help in cell lysis? Is it necessary to prepare a cell lysate before proceeding with the protocol?

There is no need to prepare a cell lysate. The Lipid extraction solution can be used according to the protocol for this purpose. However, for tissues, flash frozen, pulverized tissue can be used for lipid extraction. Otherwise, minced fresh tissue can be homogenized in the lipid extraction solution before heating the sample for lipid extraction.

Can cholesterol be assayed using this kit?

For measuring cholesterol in samples, we suggest using one of our cholesterol assays: Cholesterol/ Cholesteryl Ester Quantification Kit (Colorimetric/Fluorometric) (ab65369) or Cholesterol/Cholesteryl Ester Detection Kit (Colorimetric) (ab102515). This product measures total lipids in a sample.

Is this kit appropriate for measuring lipids in blood or other body fluids?

The Adipogenesis Kit is usually used with adipocytes or adipose tissue, and very rarely with body fluids since there is very little adipogenesis occurring in fluids. A larger volume of sample might be required to test aqueous body fluids with this kit.

Can this kit be used for testing lipid accumulation in hepatocytes from fatty liver?

Yes, liver tissue can be homogenized in the lipid extraction solution and the lipids can be assayed for.

18. **INTERFERENCES**

19. **NOTES**

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp