Adipogenesis Assay Kit

(Catalog #K610-100; 100 assays; Store at -20°C)

I. Introduction:

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. BioVision's adipogenesis assay kit 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 (λmax = 570 nm) and fluorescence (Ex/Em = 535/587 nm). The kit can detect triglyceride in as few as a thousand or less differentiated 3T3-L1 cells with triglyceride detection linear range 0.2 to 10 nmol. The high detection sensitivity and the convenient microplate assay format make the kit a convenient tool for studying the effect of adipogenesis inducers or inhibitors, or to screen drugs.

II. Kit Contents:

Components	K610-100	Cap Code	Part Number
Adipogenesis Assay Buffer	25 ml	WM	K610-100-1
Lipid Extraction Solution	10 ml	NM	K610-100-2
Adipogenesis Probe (in DMSO solution)	200 µl	Red	K610-100-3a
Lipase (lyophilized)	1 vial	Blue	K610-100-4
Adipogenesis Enzyme Mix (lyophilized)	1 vial	Green	K610-100-5
Triglyceride Standard (1 mM)	0.3 ml	Yellow	K610-100-6

III. Reagent Preparation and handling:

Store kit at –20°C, protect from light. Warm Adipogenesis Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay. Use the kit within two months after reconstitution.

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

Adipogenesis Probe: Warm to room temperature to melt frozen DMSO, which is then ready to use.

Lipase and Enzyme Mix: Reconstitute each vial with 220 µl Assay Buffer. Aliquot and store at –20°C. Keep on ice while in use.

VI. Assay Protocol:

1. Sample Preparation:

a. For microplate cultured 3T3 cells: Culture cells in a 96-well plate, treat cells with desired reagents and methods. When the cells are ready for triglyceride testing, remove medium completely from wells and wash once with PBS. Add 100 µl Lipid Extraction Solution per well, seal plate with an adhesive cover to prevent evaporation. Place entire plate* in plate heater or heating block at 90-100°C for 30 min. Solution in the wells will become cloudy when heated. Cool plate to room temperature. Mix solution by shaking plate for 1 min. Triglycerides are now completely dissolved in the Lipid Extraction Buffer.

b. **For tissue (0.1-10 mg) or cells (1,000-1 million):** Homogenize samples in 100 µl Lipid Extraction Solution then heat/vortex as described above. Centrifuge briefly (top speed using a bench top centrifuge) to remove debris/insoluble material. If oil droplets are still observed, reduce the number of cells used per assay. In our hands, ~1,000-10,000 differentiated 3T3 cells using 100 µl Lipid Extraction Solution are sufficient for the colorimetric assay.

For triglyceride assay, transfer 5-50µl** of the lipid extracts to 96-well plate, bring the volume to total 50 µl with Assay Buffer.

Notes:

*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.

**3T3 cells can accumulate exceedingly large amounts of triglyceride. Fully differentiated cells can contain 100X the amount of triglyceride as uninduced cells. The amount of the lipid extract used for the triglyceride assay will depend on cell type, treatment and cell differentiation stage. For unknown samples, we suggest testing different doses of your sample to make sure the readings are within the standard curve range.

**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 the BCA method for the protein assay.

2. Standard Curve Preparation:

For the colorimetric assay, dilute 40 µl of the 1 mM Triglyceride standard into 160 µl Assay Buffer, mix to generate 0.2 mM 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 Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of Triglyceride Standard.

For the fluorometric assay, dilute the Triglyceride Standard 10 fold further with the Triglyceride Assay Buffer, then follow the procedure as the colorimetric assay.

3 **Lipase:** Add 2 µl of lipase to each well with sample and standard. Mix and incubate 10 min at room temperature to convert triglyceride to glycerol and fatty acid.

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 Adipogenesis Assay Buffer
2 µl Probe***
2 µl Enzyme Mix

Add 50 µl of the Reaction Mix to each well containing the Triglyceride Standard, samples and controls. Mix well. Incubate at 37°C for 30 min, protect from light.

***Note:** Detection sensitivity is 10-100 fold higher for a fluorometric assay. For the fluorometric assay, use 10% of the Probe to decrease the background readings, therefore increasing detection sensitivity.

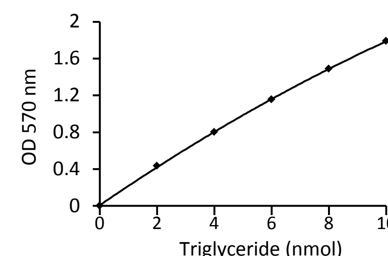
5. Measure OD 570 nm for colorimetric assay (or Ex/Em = 535/590 nm for fluorometric assay) in a plate reader.

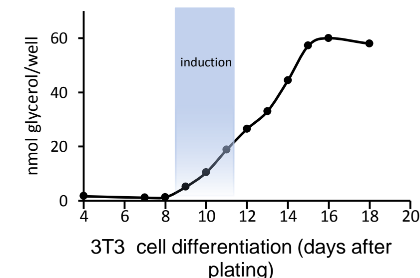
6. **Calculations:** Correct background by subtracting the value derived from the 0 triglyceride standard from all readings. Plot the standard curve. Apply sample readings to the standard curve. Triglyceride concentration can then be calculated:

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).

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





3T3 cell differentiation (days after plating)

RELATED PRODUCTS:

- Apoptosis Detection Kits & Reagents
- Cholesterol, LDL/HDL Assay Kits
- Ethanol and Uric Acid Assay Kits
- Lactate Assay Kit
- Triglyceride Assay Kit
- Glutathione Assay Kits
- NAD/NADH and NADP/NADPH Assay Kit
- Pyruvate 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 • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • 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.		

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