Ver.251101

Lipolysis Colorimetric Assay Kit

OLC102-01 (100Tests)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Lipolysis is the hydrolysis of triglycerides within the cell into glycerol and free fatty acids. The glycerol and free fatty acids are then released into the bloodstream or culture media. Lipolysis occurs in essentially all cells, but is most abundant in white and brown adipose tissue. Deficiencies in lipolysis lead to increased intracellular lipid accumulation, resulting in abnormal cellular physiology, hyperlipidemia, and insulin resistance. Lipolysis can be induced by catecholamine and certain hormones. The kit includes synthetic catecholamine, Isoproterenol, which activates β-adrenergic receptors. This leads to activation of adenylate cyclase, which catalyzes the conversion of ATP to cAMP. cAMP then serves as a second messenger to activate hormone-sensitive lipase, which hydrolyzes the triglycerides. This pathway can be inhibited by insulin.

Kit components

Reagent	Volume	Storage
Enzyme mix VI	1Vial	-20°C
Buffer IV	17mL	-20°C
Assay Buffer V	25mL	-20°C
Probe	200µL	-20°C
Glycerol Standard	200µL	-20°C
Wash Buffer VIII	22mL	-20°C
Isoproterenol	50µL	-20°C

Storage:

Store kit at -20°C in the dark immediately on receipt and check below storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted. Reconstituted components are stable for 2 months. Aliquot components in working volumes before storing at the recommended temperature. Avoid repeated freeze thaws of reagents.

Reagents and Equipment Required but Not Provided

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

- Distilled water
- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- Centrifuge with cooling option

Precaution

- To avoid contamination, use clean laboratory wares. Use clean, dry disposable pipette tips for dispensing. Close reagent bottles immediately after use.
- Avoid direct exposure of reagent to light. Do not blow into the reagent bottles

1. Reagent Preparation

1.1 Assay Buffer V: Aliquot and store at -20°C. Warm to room temperature before use.

1.2 Probe: Briefly warm at 37°C for 1-2 minutes to dissolve. Mix well. Store at -20°C. Use within 2 months.

1.3 Enzyme Mix VI: Reconstitute with 220µL Assay Buffer V. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Use within two months.

1.4 Isoproterenol: Warm to room temperature before use. Dilute the 10mM stock solution 1/1,000 in distilled water to make a 10µM working solution as needed. Store at -20°C. Use within two months.

1.5 Buffer IV and Wash Buffer VIII: Warm to 37°C before use. Store at 4°C or -20°C

2. Sample Preparation

Sample Preparation Grow and differentiate 3T3-L1 cells in a 96-well cell culture plate. After differentiation (lipid droplets should be visible using microscopy), gently wash cells two times with 100µL of Wash Buffer VIII. Remove Wash Buffer VIII and replace with 150µL Buffer IV. Add 1.5µL of 10µM Isoproterenol (final concentration 100nM) to wells to stimulate lipolysis. Stimulate lipolysis for 1- 3 hours or longer if desired. Collect media. Add 20-50µL of media into 96-well plate and adjust the volume to 50µL with Buffer IV. Cells can be lysed and used to normalize glycerol to cellular protein content using a BCA Protein Assay Kit (PC0020)

NOTES:

- Care should be taken while washing differentiated cells as differentiated cells are fragile and liable to detach with vigorous washing
- For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.
- Higher concentrations of Isoproterenol interfere with the assay. If using a higher concentration or measuring larger sample volume we recommend spiking the Standards with the same amount of Isoproterenol as used to stimulate the lipolysis and prepare Standard Curve.

3. Standard Curve Preparation:

3.1 Add 10µL of 100mM Glycerol Standard to 990µL of Assay Buffer V to generate 1mM Glycerol Standard and mix well.

3.2 Add 0, 2, 4, 6, 8, and 10µL of 1mM Glycerol Standard into series of wells in 96-well plate to generate 0, 2, 4, 6, 8, and 10nmol/well of Glycerol Standards.

3.3 Adjust the volume to 50µL per well with Assay Buffer V.

4. Reaction Mix:

Prepare enough Reaction Mix for the number of assays to be performed. For each well, prepare 50µL Mix containing:

	Reaction Mix(µL)
Assay Buffer V	46µL
Enzyme Mix VI	2µL
Probe	2µL

Mix the Reaction Mix well and then dd 50µL of the Reaction Mix to each well containing the Standards and test samples.

5. Measurement

- Incubate the plate at room temperature for 30 minutes protected from light.
- Measure absorbance at OD 570nm in a microtiter plate reader. The reaction is stable for at least 2 hours.

6. Calculations:

Subtract 0 Standard reading from all readings. Plot the Standard Curve. Apply the corrected sample reading to the Standard Curve to get B nmol of Glycerol amount in the sample wells.

Sample Glycerol Concentration: C = B x T/S = nmol/well

Where:

B = amount of glycerol from Standard Curve (nmol).

T = total volume of the sample (µL).

S = sample volume added into the reaction well (µL).

Glycerol molecular weight: 92.09 g/mol. Glycerol can be expressed in nmol or nmol/well; alternatively as nmol/µg protein or nmol/µg lipid.

7. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled- needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Problem	Reason	Solution
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the spin column
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/number of strokes with the Dounce homogenizer
	Too many freeze- thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standars	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit