PRODUCT INFORMATION & MANUAL

Adipogenesis Assay Kit (Fluorometric)

NBP3-24457

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Adipogenesis Assay Kit (Fluorometric)

Catalog No: NBP3-24457

Method: Fluorimetric method

Specification: 96T (Can detect 84 samples without duplication)

Measuring instrument: Fluorescence Microplate Reader

Sensitivity: 1.4 µmol/L

Detection range: 1.4-32 µmol/L

Average intra-assay CV (%): 4.0

Average inter-assay CV (%): 8.4

Average recovery rate (%): 98

▲ This kit is for research use only.
▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure triglyceride accumulation in cell and tissue samples.

▲Detection principle

Triglycerides were converted by enzyme to produce hydrolytic products, which were catalyzed by the enzyme to produce fluorescent substances. The triglyceride accumulation can be calculated by meas-uring the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Enzyme Working Solution	25 mL × 1 vial	2-8°C, 12 months, shading light
Reagent 2	Extraction Solution	50 mL × 1 vial	2-8°C, 12 months
Reagent 3	Probe	0.5 mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	1 mmol/L Standard	0.5 mL × 1 vial	2-8°C, 12 months, shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Fluorescence microplate reader (Ex/Em=535 nm/587 nm), Micropipettor, Incubator, Water bath

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

It is necessary to wash the preparation equipment several times before preparation of the chromogenic working solution to prevent impurity contamination.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. The preparation of chromogenic working solution:
   Mix the reagent 1 and reagent 3 at the ratio of 49: 1. Prepare the fresh needed amount before use and prepared solution should be stored with shading light.
3. The preparation of 40 μmol/L standard solution:
   Mix the reagent 4 and reagent 2 at the ratio of 1: 24. The prepared solution can be stored at 2-8℃ with shading light for 3 days.

▲ Sample preparation

1. Cell sample:

1) Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment.
2) Add homogenization medium at a ratio of cell number (10^4): reagnet 2 (μL) =1: 200.
3) Centrifuge at 10000 g at 4°C for 10 min. Take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).
4) Heat the supernatant at 90 –100°C for 30 min to ensure the lipids completely dissolved in reagent 2. Cool to room temperature for detection.

2. Tissue sample:

1) Take 0.02 g fresh tissue to wash with PBS (0.01 M, pH 7.4) at 2-8°C.
2) Add 180 μL of reagent 2 to homogenize. Centrifuge at 10000 g at 4°C for 10 min. Take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.
3) Place on ice for 30 min.
4) Heat the supernatant at 90 –100°C for 30 min to ensure the lipids completely dissolved in reagent 2. Cool to room temperature for detection. (If there is turbidity, it is recommended to centrifuge again and take the supernatant for determination). (The lipid content of the tissue sample is relatively high, so it is recommended to take 2~3 samples to do a pre-experiment and choose an appropriate dilution ratio for dilution. The diluent is reagent 2.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (1.4–32 μmol/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Porcine heart tissue homogenate	3-6
10% Porcine liver tissue homogenate	8-10
CHO cell (10^6)	1
293T cell (10^6)	1

[Note]: The diluent is reagent 2.

Assay protocol

▲Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
B	B	B	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
C	C	C	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
D	D	D	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80
E	E	E	S9	S17	S25	S33	S41	S49	S57	S65	S73	S81
F	F	F	S10	S18	S26	S34	S42	S50	S58	S66	S74	S82
G	S1	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75	S83
H	S2	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76	S84

[Note]: A-F, standard wells; S1-S84, sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 40 μmol/L standard with reagent 2 to a serial concentration. The recommended dilution gradient is as follows: 0, 8, 16, 20, 24, 32 μmol/L. Reference is as follows:

Number	Standard concentrations (μmol/L)	40 μmol/L standard (μL)	Reagent 2 (μL)
A	0	0	200
B	8	40	160
C	16	80	120
D	20	100	100
E	24	120	80
F	32	160	40

2. The measurement of samples

1) Standard well: Add 20 μL of standard with different concentrations to the corresponding wells.
   Sample well: Take 20 μL of sample supernatant to the corresponding wells.
2) Add 200 μL chromogenic working solution to each well.
3) Mix fully and incubate at 37°C for 5 min.
4) Measure the fluorescence intensity of each well at the excitation wavelength of 535 nm and the emission wavelength of 587 nm.

▲Summary operation table

	Standard well	Sample well
Sample (μL)		20
Standard with different concentrations (μL)	20	
Chromogenic working solution (μL)	200	200
Mix fully and incubate at 37°C for 5 min. Measure the fluorescence intensity of each well.		

▲Calculation

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the fluorescence value of sample.

The standard curve is: y= ax + b.

Triglyceride content (μmol/gprot)= (ΔF- b) ÷a ÷ C_{pr} × f

Note:

y: $F_{Standard}$ – F_{Blank}. (F_{Blank} is the fluorescence value when the standard concentration is 0)
x: The concentration of standard;
a: The slope of standard curve;
b: The intercept of standard curve;
ΔF: F_{Sample}– F_{Blank}. (F_{Blank} is the fluorescence value when the standard concentration is 0)
C_{pr}: Concentration of protein in sample, gprot/L.
f: Dilution factor of sample before test.

Appendix I Data

▲Example analysis

For porcine heart tissue, take 10% tissue homogenate supernatant, dilute for 5 times, then carry the assay according to the operation steps.

The results are as follows:

standard curve: y = 235.98 x - 8.5102, the average fluorescence value of the sample well is 9614, the average fluorescence value of the blank well is 2121, the concentration of protein is 2.56 gprot/L and the calculation result is:

$$\text{Triglyceride content (μmol/gprot)} = (9614 - 2121 + 8.5102) \div 235.98 \div 2.56 \times 5$$
$$= 62.08\ \text{μmol/gprot}$$