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CheKine™ Micro Fructose-1,6 Diphosphate (FDP) Content Assay Kit

Cat #: KTB1328 Size: 48 T/96 T

	Micro Fructose-1,6 Diphosphate (FDP) Content Assay Kit		
REF	**Cat #:** KTB1328	LOT	**Lot #:** Refer to product label
	Applicable samples: Animal and Plant Tissue, Cells, Plasma, Serum		
	Storage: Stored at -20℃ for 6 months, protected from light		

Assay Principle

Fructose-1,6-diphosphate (FDP) is an important intermediate product in the glycolysis process. It can regulate a variety of enzymes, improve cell energy metabolism, increase energy utilization, anti-arrhythmia and anti-tissue peroxidation. FDP is widely used in clinical medicine. CheKine™ Micro Fructose-1,6 Diphosphate (FDP) Content Assay Kit can be used to detect biological samples such as animal tissue, bacteria or cells, serum or plasma. In the kit, aldolase catalyzes the cleavage of fructose 1,6-diphosphate. The product reacts with 2,4- dinitrophenylhydrazine in acid medium to form 2, 4-dinitrophenylhydrazone, which is dark red in alkaline solution and has a characteristic absorption peak at 540 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Reagent Ⅰ	55 mL	110 mL	4℃
Reagent Ⅱ	1	1	4℃, protected from light
Reagent Ⅲ	2 mL	4 mL	4℃, protected from light
Reagent Ⅳ	8 mL	16 mL	4℃
Standard	1	1	-20℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 540 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips, 1.5 mL EP tube
- Incubator, ice maker, freezing centrifuge
- Deionized water
- Homogenizer or mortar (for tissue samples)

Reagent Preparation

Reagent I: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Reagent II: Prepared before use. Add 0.2 mL deionized water for 48 T and 0.4 mL eionized water for 96 T to fully dissolve. The prepared reagent can be stored at 4℃, protected from light for 1 month.

Reagent III: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃, protected from light.

Reagent IV: Ready to use as supplied; Equilibrate to room temperature before use; Store at 4℃.

Standard: Prepared before use; Add 1 mL deionized water to fully dissolve, that is 1 mg/mL fructose-1, 6 diphosphate standard; Store at 4℃, protected from light for 1 month.

Standard preparation: Use the 1 mg/mL fructose-1, 6 diphosphate standard solution and further dilute it to the standard as shown in the following table:

Num.	Standard Volume (μL)	Deionized Water (μL)	Concentration (μg/mL)
Std.1	100 μL 1 mg/mL Standard	100	500
Std.2	100 μL of Std.1 (500 μg/mL)	100	250
Std.3	100 μL of Std.2 (250 μg/mL)	100	125
Std.4	100 μL of Std.3 (125 μg/mL)	100	62.5
Std.5	100 μL of Std.4 (62.5 μg/mL)	100	31.25
Std.6	100 μL of Std.5 (31.25 μg/mL)	100	15.625
Std.7	100 μL of Std.6 (15.625 μg/mL)	100	7.8125

Notes: Always prepare fresh Standards per use; Diluted Std. solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend that you use fresh samples. If not assayed immediately, samples can be stored at -80℃ for one month.When measuring, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

1. Tissues: Weigh 0.1 g tissue, add 1 mL Reagent Ⅰ and homogenize or mortar on ice. Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
2. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Reagent Ⅰ to ultrasonically disrupt the cells or bacteria 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 10,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Plasma, Serum: Test directly.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 540 nm. Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement. (The following operations are operated in the 96-well plate or microglass cuvette)

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Sample	0	0	20
Standard	0	20	0
Deionized water	20	0	0

Reagent Ⅰ	44	40	40
Reagent Ⅱ	0	4	4
Mix well, incubate for 30 min at 37℃.			
Reagent Ⅲ	40	40	40
Mix well, incubate for 30 min at 37℃.			
Reagent Ⅳ	160	160	160

3. Mix well, incubate for 30 min at 37℃. Detect the absorbance at 540 nm. The Blank Well is recorded as A_{Blank}, the standard Well is marked as $A_{Standard}$, the Test Well is marked as A_{Test}. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: The Blank Well and the Standard Well only need to be done 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.02, increase the sample quantity appropriately. If ΔA_{Test} is greater than 0.5, the sample can be appropriately diluted with corresponding Extraction Buffer, the calculated result multiplied by Reagent Ⅰ, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the x-axis and the $\Delta A_{Standard}$ as the y-axis, draw the standard curve and obtain the standard equation y=kx+b. The determination of ΔA_{Test} is brought into the equation to get x (μg/mL).

2. Calculation of the FDP content

(1) Calculated by fresh weight of samples

FDP (μg/g fresh weight)=$x\div(W\div V_{Total\ sample})$**=x÷W**

(2) Calculated by bacteria or cells

FDP (μg/10^4 cell)=$x\div(n\div V_{Total\ sample})$**=x÷n**

(3) Calculated by volume of liquid samples

FDP (μg/mL)=$x\div V_{Total\ sample}$**=x**

$V_{Total\ sample}$: Added the Reagent Ⅰ volume, mL; W: Sample weight, g; n: Number of cells, calculated in units of ten thousand.

Typical Data

The following data are for reference only. And the experimenters need to test the samples according to their own experiments.

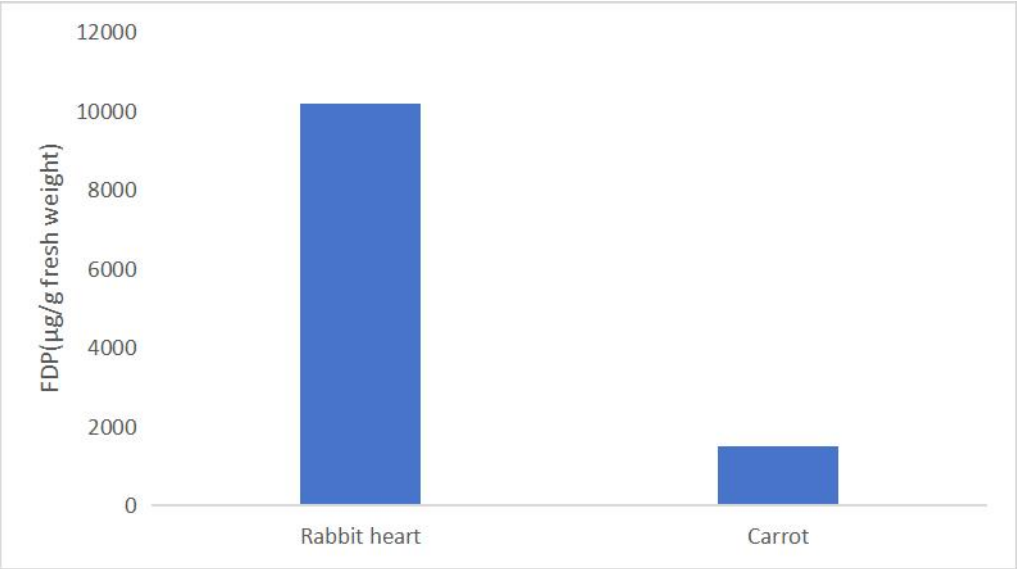

Figure 1. Determination of FDP content in rabbit heart and carrot by this kit.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Content Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.