Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

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

Cat #: KTB1328 Size: 48 T/48 S 96 T/96 S

| | | | |
|---|---|---|---|
| | **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 | 65 mL | 65×2 mL | 4℃ |
| Reagent Ⅱ | Powder×1 vial | Powder×1 vial | 4℃, protected from light |
| Reagent Ⅲ | 2.8 mL | 5.6 mL | 4℃, protected from light |
| Reagent Ⅳ | 11.2 mL | 22.4 mL | 4℃ |
| Standard | Powder×1 vial | Powder×1 vial | -20℃, protected from light |

**Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.**

## 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 0.28 mL deionized water for 48 T and 0.56 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.

**Note: Reagent III is toxic, so it is recommended to experiment in a fume hood.**

**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 $5\times10^6$ 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 10 min at 37℃. | | | |
| Reagent Ⅳ | 160 | 160 | 160 |

3. Mix well, incubate for 10 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 $\Delta A_{Test}$ is less than 0.02, increase the sample quantity appropriately. If $\Delta A_{Test}$ is greater than 0.5, the sample can be further diluted with Reagent Ⅰ, and the calculation results should be multiplied by the dilution factor. Alternatively, reduce the amount of sample used for extraction.**

## 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. The determination of $\Delta A_{Test}$ 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.

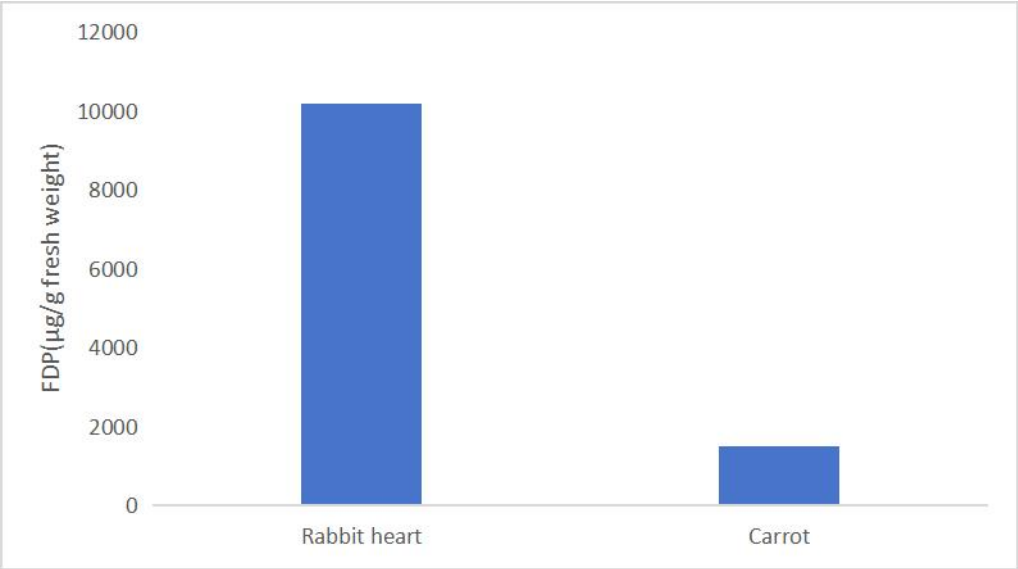


Figure 1. Determination of FDP content in rabbit heart and carrot by this kit.

## 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. For your safety and health, please wear a lab coat and disposable gloves.