

# Fructose-1, 6-diphosphate (FDP) Activity Assay Kit

**Note:** Take two or three different samples for prediction before test.

**Operation Equipment:** Spectrophotometer/ Microplate reader

**Cat No:** NA0470

**Size:**100T/48S

## Components:

Extract solution I: Liquid 60 mL×1. Store at 4°C.

Extract solution II: Liquid 10 mL×1. Store at 4°C.

Reagent I: 10 mL×1. Store at 4°C.

Reagent II: 10 μL×1×1. Store at 4°C. Dissolve with 0.209 mL of distilled water before use. Unused reagent can store at 4°C for one week.

Reagent III: 7 mL×1. Store at 4°C.

Reagent IV: 20 mL×1. Store at 4°C.

Standard: Powder×1. Store at 4°C. Dissolve with 1.176 mL of distilled water before use to form 50 μmol/mL FDP standard solution

## Product Description:

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.

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.

## Reagents and Equipment Required but Not Provided:

spectrophotometer/Microplate reader, desk centrifuge, adjustable transferpettor, water bath /incubator, micro glass cuvette/ 96 well flat-bottom plate, mortar / homogenizer, ultrasonic crusher, ice and distilled water.

## Procedure:

### I. Sample preparation:

#### 1) Tissue

According to the tissue weight (g): the volume of the extract (mL) is 1:5 ~ 10. Suggest adding 1 mL of Extract solution I to 0.1 g of tissue, fully homogenize on ice bath. Centrifuge at 12000 ×g for 10 minutes at 4°C. Take 0.8 mL of supernatant and 0.16 mL of Extract solution II to mix well, centrifuge at 12000 ×g for 10 minutes at 4°C. Then take supernatant for test.

#### 2) Bacteria or cells

According to the Bacteria or cells ( $10^4$ ): the volume of the extract (mL) is 500~1000:1. Suggest add 1mL of Extract solution to 5 million of bacteria or cells. Use ultrasonication to splitting bacteria and cells (placed on ice, ultrasonic power 300W, working time 3 seconds, interval 7 seconds, total time for 3 min). Centrifuge at  $12000 \times g$  for 10 minutes at  $4^\circ\text{C}$ . Take 0.8mL supernatant and 0.16mL Extract solution II to mix well, centrifuge at  $12000 \times g$  for 10 minutes at  $4^\circ\text{C}$ . Then take supernatant for test.

### 3) Liquid:

Add 1 mL of Extract solution I to 100 $\mu\text{L}$  liquid sample, centrifuge at  $12000 \times g$  for 10 minutes at  $4^\circ\text{C}$ . Take 0.8mL supernatant and 0.16mL Extract solution II to mix well, centrifuge at  $12000 \times g$  for 10 minutes at  $4^\circ\text{C}$ . Then take supernatant for test.

## II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust the wavelength to 540 nm, set zero with distilled water.
2. 50  $\mu\text{mol/ml}$  fructose-1,6-diphosphate standard solution is diluted to 3.125, 1.5625, 0.78125, 0.39, 0.2 and 0.1  $\mu\text{mol/ml}$  standard solution with distilled water.

### 3. Sampling table:

Reagent name ( $\mu\text{L}$ )	Control tube ( $A_C$ )	Test tube ( $A_T$ )	Blank tube ( $A_B$ )	Standard tube ( $A_S$ )
Sample	20	20	-	-
Distilled water	-	-	20	20
Standard solution			-	20
Reagent I	44	44	44	40
Reagent II	-	4	-	4
Mix well, react accurately at $37^\circ\text{C}$ for 2 h				
Reagent III	40	40	40	40
Mix well, react accurately at $37^\circ\text{C}$ for 20 min				
Reagent IV	100	100	100	100
Mix well, react accurately at $37^\circ\text{C}$ for 10 min				
The absorbance value at 540 nm is measured in 1 mL glass cuvette and recorded as $A_C$ , $A_T$ , $A_B$ , $A_S$ , respectively. Calculate $\Delta A = A_T - A_C$ , $\Delta A_S = A_S - A_B$ . The blank tube only needs to be tested 1-2 times.				

## III. Calculation:

1. According to concentration of standard solution and  $\Delta A_S$  to create the standard curve, take standard solution as X-axis,  $\Delta A_S$  as Y-axis. Take  $\Delta A$  into the equation to obtain x ( $\mu\text{mol/ml}$ ).

### 2. Calculation:

#### (1) sample weight

$$\text{FDP (mg/g fresh weight)} = x \times (V_{\text{su}} + V_{\text{exII}}) \times M \div (W \times V_{\text{su}} \div V_{\text{exI}}) = 408x \div W$$

#### (2) The number of bacteria or cells

$$\text{FDP (mg/}10^4\text{ cell)} = x \times (V_{\text{su}} + V_{\text{exII}}) \times M \div (\text{cell amount} \times V_{\text{su}} \div V_{\text{exI}}) = 408x \div \text{cell amount}$$

#### (3) Liquid:

$$\text{FDP (}\mu\text{mol/mL)} = x \times (V_{\text{su}} + V_{\text{exII}}) \div (V_L \times V_{\text{su}} \div (V_{\text{exI}} + V_L)) = 13.2x$$

$V_{su}$ : Supernatant volume of extraction, 0.8mL

$V_{exII}$ : Extract solution II volume, 0.16mL

M: Molecular weight of fructose-1,6-diphosphate, 340

$V_{exI}$ : Extract solution I volume, 1mL

W: sample weight, g

Cell amount: 10 thousand cells as unit

$V_L$ : liquid sample volume, 0.1mL.

**Note:**

1. If  $\Delta A > 0.5$ , please dilute the sample with water to appropriate concentration, multiply dilute times in the formula.

**Related Products:**

NA0708/NA0467 Fructose-bisphosphate aldolase(FBA) Activity Assay Kit

NA0710/NA0469 Phosphoglycerate Kinase(PGK) Activity Assay Kit