SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

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: AK0239

Size:100T/48S

Components:

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

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

Reagent I: 10 mL×1. Store at 4℃ .

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

Reagent III: 7 mL×1. Store at 4℃ .

Reagent IV: 20 mL×1. Store at 4℃ .

Standard: Powder×1. Store at 4℃ . 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℃ . 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℃ . 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 ×g for 10 minutes at 4℃ . Take 0.8mL supernatant and 0. 16mL Extract solution II to mix well, centrifuge at 12000 ×g for 10 minutes at 4℃ . Then take supernatant for test.

3) Liquid:

Add 1 mL of Extract solution I to 100μL liquid sample, centrifuge at 12000×g for 10 minutes at 4℃ . Take 0.8mL supernatant and 0. 16mL Extract solution II to mix well, centrifuge at 12000×g for 10 minutes at 4℃ . 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 μmol/ml fructose- 1,6-diphosphate standard solution is diluted to 3. 125, 1.5625, 0.78125, 0.39, 0.2 and 0. 1μmol/ml standard solution with distilled water.

3. Sampling table:

Reagent name (μL)	Control tube (A_C)	Test tube (A_T)	Blank tube (A_B)	Standard tube (As)
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℃ for 2 h				
Reagent III	40	40	40	40
Mix well, react accurately at 37℃ for 20 min				
Reagent IV	100	100	100	100
Mix well, react accurately at 37℃ 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 ΔA_S to create the standard curve, take standard solution as X-axis, ΔAs as Y-axis. Take ΔA into the equation to obtain x (μmol/ml).

2. Calculation:

(1) sample weight

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

(2) The number of bacteria or cells

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

(3) Liquid:

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

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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 ΔA>0.5, please dilute the sample with water to appropriate concentration, multiply dilute times in the formula.

Related Products：

AK0238/AK0237	Fructose-bisphosphate aldolase(FBA) Activity Assay Kit
AK0394/AK0393	Phosphoglycerate Kinase(PGK) Activity Assay Kit