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Fructose-1,6-bisphosphate aldolase (FBA) Activity Assay Kit (UV method)

Product Number: BC2271

Shipping and Storage

1. Reagent 1 and Reagent 2 : Store at -20℃;
2. Reagent 3: Store at -20℃ away from light;
3. Extraction Solution, Reagent 4 and Reagent 5: Store at 4℃.
4. Validity period: 3 months.

Component

Component	48T
Extraction Solution	60mL× 1 bottle
Reagent 1	Liquid, 1 bottle
Reagent 2	Powder, 4 vials
Reagent 3	Liquid, 1 bottle
Reagent 4	30mL × 1 bottle
Reagent 5	Powder × 1 bottle

1. Reagent 1: Tap or centrifuge the vial before opening to collect all reagent at the bottom; Add 2.1mL of distilled water for reconstitution prior to use; Valid for the same shelf- life as the kit.
2. Reagent 2: For each vial: Centrifuge at 8000 g, 4℃ for 2 min before use to pellet powder to the tube bottom. Add 0.6mL distilled water to dissolve. Aliquot unused portions and store at −20℃. Avoid repeated freeze-thaw cycles. Use within 3 days after reconstitution.
3. Reagent 3: Tap or centrifuge the vial before opening to collect all reagent at the bottom. Add 2.2mL distilled water for reconstitution prior to use. Valid for the same shelf- life as the kit.
4. Reagent 5: Tap or centrifuge the vial before opening to collect all powder at the bottom. Add 4.2mL distilled water for reconstitution prior to use. Valid for the same shelf- life as the kit.

Description

Fructose-1,6-bisphosphate aldolase (EC 4.1.2.13, FBA) participates in glycolysis/gluconeogenesis as well as the pentose phosphate cycle. It supplies ATP energy and substrates for biosynthetic metabolism in organisms, and therefore plays a critical role in cellular vital activities.

FBA catalyzes the cleavage of fructose-1,6-bisphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Under sequential action of coupled enzymatic complexes, NADH and dihydroxyacetone phosphate are converted into NAD^+ and α-glycerophosphate. FBA activity is determined by monitoring the decreasing rate of NADH absorbance at 340nm.

Required Laboratory Equipment

Mortar (homogenizer), ice box (ice-maker), table-top centrifuge, adjustable pipettors, water bath (oven / incubator / metal heat block), 1mL quartz cuvettes, centrifuge tubes, UV-visible spectrophotometer, distilled water (deionized water or ultrapure water is acceptable).

Sample Assay

It is recommended to run a pilot test with 1-3 representative samples of distinct groups/types to get familiar with the workflow. Adjust sample loading concentration according to pilot results to avoid unnecessary consumption of samples and reagents.

1. **Sample Extraction**

1.1. Tissue Samples

Weigh 0.1g tissue sample, add 1 mL Extraction Solution, and perform ice-bath homogenization. Centrifuge homogenate at 12 000 rpm, 4℃ for 10 min. Collect supernatant for subsequent measurement.

Note: For larger sample input, perform extraction at a mass-to-volume ratio: tissue weight (g) : Extraction Solution volume (mL) = 1 : 5 ~ 10.

1.2. Bacterial / Cell Samples

Harvest bacteria or cultured cells into centrifuge tubes and discard supernatant after centrifugation. Take 5 million bacterial or mammalian cells and add 1mL Extraction Solution. Sonicate on ice (power output: 20 % or 200 W; sonicate 3 s, interval 10 s; repeat for 30 cycles). Centrifuge lysate at approximately 12 000 rpm, 4 ℃ for 10 min. Collect supernatant as test sample.

Note: For larger sample input, perform extraction at ratio: cell count ($\times 10^4$ cells) : Extraction Solution (mL) = 500~1000 :1. Cell lysis using commercial lysis buffer is also acceptable; follow the lysis-buffer manufacturer's instructions.

2. **Protocol**

2.1. Pre-warm UV spectrophotometer for 30 min. Set wavelength to 340nm and temperature to 25 ℃.

2.2. Thaw all reagents to room temperature (25 ℃).

2.3. Pipette reagents sequentially into a 1mL quartz cuvette (1 cm light-path):

Component	Assay tube
Sample	80μL
Reagent 1	40μL
Reagent2	40μL
Reagent 3	40μL
Reagent 4	520μL
Reagent 5	80μL
Mix gently. Record absorbance A1 at 1 min and absorbance A_2 at 5 min at 340nm under 25 ℃. Calculate ΔA = A1 − A2.	

Notes:

If ΔA is close to zero, extend reaction time to 15 min before reading A2. Substitute the new incubation time into calculation formula. Alternatively, increase sample loading volume and apply the adjusted volume in calculation.

If absorbance decay is non-linear, record absorbance every 10 seconds, select a linear decreasing interval for calculation, and substitute corresponding absorbance values and time into formula.

If initial absorbance A1 is excessively high (> 2, e.g. pigment-rich plant leaf samples): reduce sample loading volume and substitute revised volume for calculation. Or add activated charcoal to sample supernatant, incubate for 5 min, centrifuge at 12 000 rpm, 4 ℃ for 10 min, collect supernatant for assay.

If ΔA > 0.6, shorten reaction time (e.g. 2 min). Substitute revised reaction time T for calculation.

Calculation of Results

1. Calculation based on sample protein concentration:

Enzyme-activity definition: One unit of enzyme activity is defined as consumption of 1nmol NADH per minute per milligram of tissue protein.

FBA activity (nmol/min/mg prot) = [ΔA ÷ (ε × d) × V2 × 10^9] ÷ (V1× Cpr) ÷ T = 321.54 × ΔA ÷ Cpr

2. Calculation based on sample fresh weight

Enzyme-activity definition: One unit of enzyme activity is defined as consumption of 1nmol NADH per minute per gram fresh-weight tissue.

FBA activity (nmol/min/g fresh weight) = [ΔA ÷ (ε × d) × V2× 10^9] ÷ (W × V1 ÷ V) ÷ T = 321.54 × ΔA ÷ W

3. Calculation based on cell number

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Enzyme-activity definition: One unit of enzyme activity is defined as consumption of 1 nmol NADH per minute per 1×10^4 cells.

FBA activity (nmol/min/10^4 cell) = [$\Delta A \div (\varepsilon \times d) \times V2 \times 10^9$] ÷ (500 × V1 ÷ V) ÷ T = 0.643 × ΔA.

Parameter explanations:

ε — Molar extinction coefficient of NADH = 6.22×10^3 L/mol/cm

d — Cuvette light-path = 1 cm

V — Volume of Extraction Solution added = 1mL

V_1 — Volume of sample loaded in reaction = 0.08mL

V_2 — Total reaction volume = 0.2 mL = 8×10^{-4} L

T — Reaction incubation time = 5 min

W — Sample fresh weight (g)

500 — Cell count unit: ten-thousand cells

Cpr — Sample protein concentration (mg/mL). BCA Protein Quantification Kit from our company is recommended for protein determination.