Acetyl Coenzyme A (Acetyl-CoA) Fluorometric Assay Kit

Cat. No. KITG-010 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit can be used to measure acetyl coenzyme A (Acetyl-CoA) content in serum (plasma), animal tissue and cell.
Description	Acetyl-CoA is an essential cofactor and acyl carrier in the enzymatic acetyl transfer reaction. It can be formed by oxidative decarboxylation of pyruvate in mitochondria, oxidation of long-chain fatty acids, or oxidative degradation of certain amino acids. Acetyl-CoA is the starting compound of the citric acid cycle. The detection principle of this kit is that Acetyl-CoA is converted into other substances under the condition of enzyme catalysis, and the resulting fluorescence is detected by fluorescence microplate reader at the maximum excitation wavelength of 535 nm and the maximum emission wavelength of 587 nm.
Form	Fluorometric Assay Kit
Bio-activity	Intra-assay Precision: Sample 1: Mean 1.50 µmol/L, %CV 1.7 Sample 2: Mean 2.50 µmol/L, %CV 1.9 Sample 3: Mean 4.00 µmol/L, %CV 4.1 Inter-assay Precision: Three rat serum were assayed 20 times in duplicate by three operators. Recovery: Average recovery rate of 97.6%. Standard 1: Expected 1.5 µmol/L, Observed 1.4 µmol/L, Recovery 92% Standard 2: Expected 2.5 µmol/L, Observed 2.4 µmol/L, Recovery 94% Standard 3: Expected 4.0 µmol/L, Observed 4.3 µmol/L, Recovery 107% Sensitivity: The analytical sensitivity of the assay is 0.27 µmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

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Applications	Measurement of acetyl coenzyme A (Acetyl-CoA) content in serum (plasma), animal tissue and cell
Notes	Kit Components & Storage: Reagent 1: Extracting Solution Size 1 (48 T): 40 mL × 1 vial Size 2 (96 T): 40 mL × 2 vials Storage: -20°C, 12 months, shading light Reagent 2: Buffer Solution Size 1 (48 T): 24 mL × 1 vial Size 2 (96 T): 48 mL × 1 vial Storage: -20°C, 12 months, shading light Reagent 3: Cofactor Size 1 (48 T): Powder × 4 vials Size 2 (96 T): Powder × 2 vials Storage: -20°C, 12 months, shading light Reagent 4: Substrate Size 1 (48 T): Powder × 4 vials Size 2 (96 T): Powder × 2 vials Storage: -20°C, 12 months, shading light Reagent 5: Enzyme Solution Size 1 (48 T): 0.5 mL × 1 vial Size 2 (96 T): 1.0 mL × 1 vial Storage: -20°C, 12 months, shading light Reagent 6: Enzyme Reagent Size 1 (48 T): 0.36 mL × 1 vial Size 2 (96 T): 0.72 mL × 1 vial Storage: -20°C, 12 months, shading light Reagent 7: Fluorescence Probe Size 1 (48 T): 3 mL × 1 vial Size 2 (96 T): 6 mL × 1 vial Storage: -20°C, 12 months, shading light Reagent 8: Standard (500 μmol/L) Size 1 (48 T): 0.5 mL × 1 vial Size 2 (96 T): 0.5 mL × 1 vial Storage: -20°C, 12 months, shading light Black Microplate: 96 wells Storage: No requirement Plate Sealer: 2 pieces Sample Layout Sheet: 1 piece Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagent. Materials Required but Not Supplied: Instruments: Fluorescence microplate reader (Ex/Em = 535 nm / 587 nm) Consumptive material: 10 kDa MWCO Spin Filter Reagent Preparation: 1. Equilibrate all reagents to 25°C before use. 2. The preparation of co-factor working solution: Dissolve one vial of co-factor with 8 mL of buffer solution, mix well to dissolve. Store at -20°C for 3 days. 3. The preparation of substrate working solution: Dissolve one vial of substrate with 7.5 mL of co-factor working solution, mix well to dissolve. Store at -20°C for 3 days. (When opening the cover, be careful not to spill the co-factor and substrate powder.) 4. The preparation of enzyme working solution: For each well, prepare 70 μL of enzyme working solution (mix well 8

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μL of enzyme solution, 6 μL of enzyme reagent and 56 μL of buffer solution). The enzyme working solution should be prepared on spot and used for same day. 5. The preparation of 5 μmol/L standard solution: Before testing, please prepare sufficient 5 μmol/L standard solution. For example, prepare 1000 μL of 5 μmol/L standard solution (mix well 10 μL of 500 μmol/L standard and 990 μL of double distilled water). Keep it on ice during use. The remaining 500 μmol/L standard can be divided to avoid repeated freezing and thawing. The stability of 5 μmol/L standard decreased after dilution, and is used within 4 hours. 6. The preparation of standard curve: Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 5 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 1, 2, 3, 3.5, 4, 4.5, 5 μmol/L. Standard curve preparation reference: Concentration (μmol/L): 0, 1, 2, 3, 3.5, 4, 4.5, 5 5 μmol/L Standard (μL): 0, 40, 80, 120, 140, 160, 180, 200 Double distilled water (μL): 200, 160, 120, 80, 60, 40, 20, 0 Sample Preparation: 1. Serum and plasma: Add 200-400 μL of serum (plasma) sample into 10 kDa MWCO Spin Filter and centrifuge at 12000×g for 20 min. Collect the filtrate and preserve it on ice for detection. Tissue sample: 1. Harvest the amount of tissue needed for each assay (initial recommendation 100 mg). 2. Wash tissue in cold PBS (0.01 M, pH 7.4). 3. Homogenize 100 mg tissue in 900 μL extracting solution with a dounce homogenizer at 4°C. 4. Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. 5. Collect 200-400 μL supernatant and add it to 10 kDa MWCO Spin Filter. Centrifuge at 12000×g for 20 min at 4°C. 6. Take the filtered sample supernatant and preserve it on ice for detection. Cell (adherent or suspension) samples: 1. Harvest the number of cells needed for each assay (initial recommendation 1 × 10^6 cells). 2. Centrifuge at 400×g for 5 min at 4°C. 3. Homogenize 1 × 10^6 cells in 200 μL extracting solution with a dounce homogenizer at 4°C. 4. Centrifuge at 10000×g for 5 min at 4°C to remove insoluble material. 5. Collect supernatant and add it to 10 kDa MWCO Spin Filter. Centrifuge at 12000×g for 20 min at 4°C. 6. Collect the filtrate and preserve it on ice for detection. 2. Dilution of sample: The recommended dilution factor for

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different samples is as follows (for reference only): Sample type and Dilution factor: 10% Mouse heart tissue homogenate: 1 10% Mouse liver tissue homogenate: 1 10% Mouse lung tissue homogenate: 1 10% Mouse kidney tissue homogenate: 1 Mouse plasma: 1 Rat serum: 1 Rat plasma: 1 1 × 10^6 CHO cells: 1 1 × 10^6 Molt-4 cells: 1 1 × 10^6 HL-60 cells: 1 Note: The diluent is extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor. The key points of the assay: The sample preparation to be tested on the ice box and should be tested within 4 hours. Operating Steps: 1. Standard well: add 20 µL of standard with different concentrations into the well. Sample well: add 20 µL of sample into the well. 2. Add 120 µL of substrate working solution into each well. 3. Add 70 µL of enzyme working solution into each well. 4. Add 50 µL of fluorescence probe into each well. 5. Mix fully with fluorescence microplate reader for 5 s. Incubate at 37°C for 10 min and measure the fluorescence intensity at the excitation wavelength of 535 nm and the emission wavelength of 587 nm. ΔF = Fsample – Fblank. Calculation: The standard curve: 1. Average the duplicate reading for each standard. 2. Subtract the mean fluorescence value of the blank (Standard #1) from all standard readings. This is the absoluted fluorescence value. 3. Plot the standard curve by using absoluted fluorescence value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL). The sample: 1. Serum and plasma samples: Acetyl-CoA content (µmol/L) = (ΔF - b) ÷ a × f 2. Tissue sample: Acetyl-CoA content (µmol/kg wet weight) = (ΔF - b) ÷ a ÷ v × f 3. Cell sample: Acetyl-CoA content (µmol/10^6) = (ΔF - b) ÷ a ÷ n × f Note: ΔF: ΔF = Fsample – Fblank. (Fblank is the fluorescence value when the standard concentration is 0). m: The weight of tissue, kg. v: The volume of extracting solution in the preparation of sample, L. n: The number of cells. f: Dilution factor of sample before test. Standard curve reference data (for reference only): Concentration (µmol/L): 0, 1, 2, 3, 3.5, 4, 4.5, 5 Fluorescence value (duplicate): 978/959, 1810/1783, 2337/2301, 2789/2816, 3112/3028, 3360/3310, 3655/3625, 3935/3842 Average fluorescence value: 968, 1797, 2319, 2803, 3070, 3335, 3640, 3888 Absoluted fluorescence value:

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	0, 829, 1351, 1835, 2102, 2367, 2672, 2920 Standard curve equation: y = 562.22 x + 143, R^2 = 0.9935 Example analysis: Take 20 μL of 10% mouse kidney tissue filtrate and carry the assay according to the operation steps. The results are as follows: Standard curve: y = 562.22 x + 143 The average fluorescence value of the sample well is 1919.06 The average fluorescence value of the blank well is 968.10 ΔF = 1919.06 – 968.10 = 950.96 Acetyl-CoA content (μmol/kg wet weight) = (950.96 - 143) ÷ 562.22 ÷ 0.1 × 0.9 = 12.93 μmol/kg wet weight Statement: 1. This assay kit is for Research Use Only. 2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiment. 3. Protection methods must be taken by wearing lab coat and latex gloves. 4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample. 5. It is recommended to take a pre-test if your sample is not listed in the instruction manual. 6. The experimental results are closely related to the situation of reagents, operations, environment and so on. The quality of the kits is guaranteed only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before experiment.
Storage	Store all reagents at -20°C, 12 months, shading light. Black Microplate: No requirement. Note: The reagents must be stored strictly according to the preservation conditions. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use.
Usage	For Research Use Only.

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