Lipoxygenase (LOX) Activity Fluorometric Assay Kit

Cat. No. KITG-144 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This kit can be used to measure lipoxygenase (LOX) activity in serum (plasma), animal tissue and cell samples. Detection range: 0.05-0.50 U/L.
Description	Lipoxygenase (LOX) is an iron-containing oxidoreductase found in plants and animals. The detection principle of this kit is as follows: LOX catalyzes the oxidation of arachidonic acid to produce fluorescence products, and the enzyme activity of LOX can be calculated according to the change of fluorescence value of fluorescence products at the excitation wavelength of 507 nm and the emission wavelength of 547 nm within a unit time.
Form	Size 48T (32 samples) / 96T (80 samples) Kit Components Buffer Solution: 7 mL × 1 vial (48T); 14 mL × 1 vial (96T). Storage: -20°C, 12 months, shading light. Substrate: 0.7 mL × 1 vial (48T); 1.4 mL × 1 vial (96T). Storage: -20°C, 12 months, shading light. Probe: Powder × 1 vial (48T); Powder × 2 vials (96T). Storage: -20°C, 12 months, shading light. Probe Solution: 0.15 mL × 1 vial (48T); 0.3 mL × 1 vial (96T). Storage: -20°C, 12 months, shading light. 1 mmol/L Standard: 0.1 mL × 1 vial (48T); 0.1 mL × 1 vial (96T). Storage: -20°C, 12 months, shading light. Black Microplate: 96 wells. Storage: no requirement. Plate Sealer: 2 pieces. Sample Layout Sheet: 1 piece.
Bio-activity	Quality Control Test Intra-assay precision: three rat serum were assayed in replicates of 20 to determine precision within an assay (CV = coefficient of variation). Mean (U/L): Sample 1: 0.09, Sample 2: 0.20, Sample 3: 0.40; %CV: Sample 1: 2.2, Sample 2: 4.9, Sample 3: 3.2. Inter-assay precision: three rat serum were assayed 20 times in duplicate by three operators to determine precision between assays. Mean (U/L):

Tel: 1-631-559-9269 1-516-512-3133
Email: info@creative-biomart.com Fax: 1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

	Sample 1: 0.09, Sample 2: 0.20, Sample 3: 0.40; %CV: Sample 1: 6.4, Sample 2: 9.4, Sample 3: 8.6. Recovery: take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 101%. Expected Conc. (U/L): Sample 1: 0.09, Sample 2: 0.20, Sample 3: 0.40; Observed Conc. (U/L): Sample 1: 0.089, Sample 2: 0.204, Sample 3: 0.404; Recovery rate (%): Sample 1: 99, Sample 2: 102, Sample 3: 101. Standard curve (reference only): as the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), the standard curve and data are provided as below for reference only. Concentration (μmol/L): 0, 2, 4, 5, 6, 7, 8, 10; F_1 values (duplicate readings): 2082/1892, 2345/2680, 2980/3350, 3325/3729, 3657/3933, 3877/4031, 4219/4420, 4819/4910; average F_1 values: 1987, 2513, 3165, 3527, 3795, 3954, 4320, 4865; absoluted F_1 values: 0, 525, 1178, 1540, 1808, 1967, 2332, 2877; standard curve equation: $y = 289.99x + 6.032$, $R^2 = 0.9967$. Sensitivity The analytical sensitivity of the assay is 0.05 U/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.
Applications	Detection Method Fluorometric assay. Measuring instrument: fluorescence microplate reader (Ex/Em = 507 nm/547 nm). Compatible Sample Types Serum (plasma), animal tissue, and cell samples (adherent or suspension).
Notes	Notes 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 reagents. The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor. Warning 1. This assay kit is for Research Use Only. We will not be responsible for any arising problems or

	legal responsibilities caused by using the kit for clinical diagnosis or other purposes. 2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments. 3. Protection methods must be taken by wearing lab coat and latex gloves. 4. If the concentration of the 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 pretest if your sample is not listed in the instruction book. 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 we are NOT 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 use.
Storage	Storage -20°C, protected from light (shading light). Stability 12 months at -20°C protected from light.
Reaction conditions	Preparation 1. Equilibrate all reagents to 25°C before use. 2. Substrate working solution: before testing, please prepare sufficient substrate working solution. For example, prepare 550 µL of substrate working solution (mix well 500 µL of buffer solution and 50 µL of substrate). The substrate working solution should be prepared on spot and used up within 1 h. 3. Probe working solution: dissolve one vial of probe with 0.09 mL of probe solution, mix well to dissolve. Store at -20°C for 2 weeks protected from light. 4. Reaction working solution: before testing, please prepare sufficient reaction working solution according to test wells. For example, prepare 505 µL of reaction working solution (mix well 500 µL of substrate working solution and 5 µL of probe working solution). The reaction working solution should be prepared on spot and used up within 1 h. 5. 10 µmol/L standard solution: before testing, please prepare sufficient 10 µmol/L standard solution. For example, prepare 1000 µL of 10 µmol/L standard solution (mix well 10 µL of 1 mmol/L standard and 990 µL of double distilled water). Store at -20°C for 2 weeks protected from light. 6. Standard curve:

always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute the 10 μmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is 0, 2, 4, 5, 6, 7, 8 and 10 μmol/L. Reference: 0 μmol/L - 10 μmol/L standard 0 μL + double distilled water 200 μL; 2 μmol/L - 40 μL standard + 160 μL water; 4 μmol/L - 80 μL standard + 120 μL water; 5 μmol/L - 100 μL standard + 100 μL water; 6 μmol/L - 120 μL standard + 80 μL water; 7 μmol/L - 140 μL standard + 60 μL water; 8 μmol/L - 160 μL standard + 40 μL water; 10 μmol/L - 200 μL standard + 0 μL water. Assay Protocol 1. Standard well: add 10 μL of standard with different concentrations into the well. Sample well: add 10 μL of sample into the well. 2. Add 140 μL of reaction working solution into each well. 3. Mix fully with fluorescence microplate for 5 s. Measure the fluorescence intensity at the excitation wavelength of 507 nm and the emission wavelength of 547 nm, recorded as F_1. 4. Incubate at 37°C for 20 min protected from light. Measure the fluorescence intensity at the excitation wavelength of 507 nm and the emission wavelength of 547 nm, recorded as F_2. (The standard curve is fitted to the standard wells in F_1.) Assay time 20 min at 37°C, protected from light (reaction time). Analysis The standard curve: 1. Average the duplicate reading for each standard. 2. Subtract the mean F_1 value of the blank (Standard #1) from all standard readings. This is the absoluted F_1 value. 3. Plot the standard curve by using the absoluted F_1 value of the standard and the correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL). Serum (plasma) samples: LOX activity (U/L) = (ΔF - b) ÷ a × f ÷ T. Tissue and cell samples: LOX activity (U/gprot) = (ΔF - b) ÷ a ÷ Cpr × f ÷ T. Note: ΔF = ΔFsample - ΔFblank (ΔFsample = F_2 - F_1, ΔFblank = F_2 - F_1). Cpr: concentration of protein in sample, gprot/L. f: dilution factor of sample before tested. T: reaction time, 20 min. Example analysis: take 10 μL of 10% mouse liver tissue homogenate which is diluted for 100 times and carry the assay according to the operating steps. The results are as follows: standard curve: y =

	289.99x + 6.032; the average F_1 value of the sample well is 2296; the average F_2 value of the sample well is 3694; the average F_1 value of the blank well is 1987; the average F_2 value of the blank well is 2110. ΔF = ΔFsample - ΔFblank = (3694 - 2296) - (2110 - 1987) = 1275. The concentration of protein is 19.70 gprot/L, and the calculation result is: LOX activity (U/gprot) = (1275 - 6.032) ÷ 289.99 ÷ 19.70 × 100 ÷ 20 = 1.11 U/gprot.
Unit definition	Serum (plasma) samples: the amount of enzyme in 1 L serum or plasma per 1 min that produces 1 μmol product at 37°C is defined as 1 unit. Tissue and cell samples: the amount of enzyme in 1 g tissue or cell protein per 1 min that produces 1 μmol product at 37°C is defined as 1 unit.
Usage	Usage Serum (plasma) samples: detect directly. Tissue samples: 1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg). 2. Wash the tissue in normal saline (0.9% NaCl). 3. Homogenize 20 mg of tissue in 180 μL of normal saline (0.9% NaCl) with a dounce homogenizer at 4°C. 4. Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Collect the supernatant and keep it on ice for detection. 5. Meanwhile, determine the protein concentration of the supernatant. Cell (adherent or suspension) samples: 1. Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells). 2. Wash the cells with normal saline (0.9% NaCl). 3. Homogenize 1×10^6 cells in 200 μL of normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4°C. 4. Centrifuge at 10,000×g for 10 min at 4°C to remove insoluble material. Collect the supernatant and keep it on ice for detection. 5. Meanwhile, determine the protein concentration of the supernatant. Dilution of sample (the recommended dilution factor for different samples is as follows, for reference only): human serum: 50-100; rat serum: 50-100; 10% mouse kidney tissue homogenate: 100-200; 10% mouse liver tissue homogenate: 100-200; 10% mouse lung tissue homogenate: 100-200; 10% mouse heart tissue homogenate: 100-200; 1×10^6 HL-60 cells: 1; 1×10^6 Jurkat cells: 1. Materials Required but Not

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	Supplied Instruments: fluorescence microplate reader (Ex/Em = 507 nm/547 nm); incubator (37°C). Reagents: normal saline (0.9% NaCl).

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