Human Cytochrome P450 CYP2C9 ELISA Quantitative Detection Kit

Cat. No. KITG-021 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Cytochrome P450 family member 2C9 (CYP2C9) is an important drug-metabolizing enzyme belonging to the CYP2C subfamily of the cytochrome P450 superfamily. CYP2C9 is widely involved in the metabolism of various drugs in the human body, including anticoagulants (such as warfarin), antiepileptic drugs (such as phenytoin), hypoglycemic drugs (such as tolbutamide), and non-steroidal anti-inflammatory drugs (such as ibuprofen).
Species	Human
Description	This kit uses a double-antibody sandwich ELISA technique. The capture antibody is coated on the enzyme-labeled plate to capture the target CYP2C9 in samples and standards. After washing, a biotin-labeled detection antibody is added for incubation, followed by washing to form a "capture antibody-antigen-detection antibody" immune complex. Subsequently, streptavidin-conjugated horseradish peroxidase (SA-HRP) is added for incubation. After incubation and washing, TMB substrate is added for color development. If the sample contains the target, the color turns blue. Stop solution is added to terminate the reaction. Free components are washed away during the detection process. The OD value is measured at 450 nm using a microplate reader. The depth of color is proportional to the content of the target in the sample. The concentration of CYP2C9 in the sample is calculated by plotting a standard curve.
Bio-activity	Recovery Rate: - Serum (n=8): Range 86-102%, Average 94% - Plasma (n=8): Range 90-106%, Average 98% - Cell Culture Supernatant (n=8): Range 94-110%,

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	Average 102% Linearity (Dilution Recovery): - Serum: 1:2 dilution 92-96%, 1:4 dilution 88-104%, 1:8 dilution 95-102% - Plasma: 1:2 dilution 90-98%, 1:4 dilution 89-107%, 1:8 dilution 92-101% - Cell Culture Supernatant: 1:2 dilution 92-112%, 1:4 dilution 104-116%, 1:8 dilution 99-115%
Purity	This kit recognizes recombinant human CYP2C9. No significant cross-reactivity was observed with other related proteins prepared at 50 ng/mL in dilution buffer.
Applications	Quantitative detection of human CYP2C9 in cell culture supernatant, serum, and plasma. Suitable for research in drug metabolism, pharmacokinetics, and clinical pharmacology studies.
Notes	1. For research use only. Not for use in diagnostic procedures. 2. The shelf life of the kit must not exceed the expiration date on the kit label. 3. Do not mix or substitute reagents with those from other batches or sources. 4. If samples produce values higher than the highest standard, dilute the samples with assay diluent and repeat the assay. 5. Changes in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in results. 6. Variations in sample collection, processing, and storage may cause differences in sample values. 7. The kit is designed to eliminate potential interfering factors from different biological samples, but cannot cover all potential interfering factors. Other interferences cannot be excluded. 8. When mixing protein solutions, always avoid foaming. 9. To avoid cross-contamination, change pipette tips when adding each standard, sample, and reagent. In addition, each reagent should be used in a separate container. 10. Ensure reagents are added continuously to the wells. To ensure accurate results, the plate sealer must be firmly attached during incubation steps. 11. When using an automatic plate washer, add a 30-second soaking period after adding wash buffer, or rotate the plate 180 degrees between wash steps to improve assay precision. 12. The chromogen should remain colorless until added to the plate. Ensure the chromogen is protected from light. The chromogen should

	change from colorless to blue. 13. The stop solution should be added to the plate in the same order as the chromogen. After adding stop solution, the color in the wells will change from blue to yellow. Green-colored wells indicate that the stop solution has not been thoroughly mixed with the substrate solution. 14. The stop solution provided in this kit is a dilute sulfuric acid solution with certain corrosiveness; handle with care. 15. Some components in this kit contain preservatives that may cause skin allergic reactions; wear a mask to avoid inhaling mist. 16. Chromogen B may cause skin, eye, and respiratory irritation; wear a mask to avoid inhaling mist. 17. Wear protective gloves, protective clothing, eye and face protection. Wash hands thoroughly after handling.
Storage	Pre-coated ELISA Plate (8×6 strips for 48T, 8×12 strips for 96T): 2-8°C Standard (1 vial): 2-8°C 100× Biotinylated Antibody (1 vial): 2-8°C 100× SA-HRP (1 vial): 2-8°C 20× Concentrated Diluent (1 vial): 2-8°C Chromogen A (1 vial): 2-8°C Chromogen B (1 vial): 2-8°C Stop Solution (1 vial): 2-8°C 20× Concentrated Wash Buffer (1 vial): 2-8°C Plate Sealer (2 pieces): No requirement Product Manual (1 copy): Please read carefully
Concentration	Specification: 96T / 48T Detection Range: 0.625–40 ng/mL Sensitivity: 0.31 ng/mL Intra-assay CV: <10% Inter-assay CV: <10%
Reaction conditions	Materials Required: 1. Microplate reader with 450 nm wavelength; 600-680 nm correction wavelength is preferred. 2. Pipettes and tips. 3. Distilled or deionized water. 4. 100-1000 mL graduated cylinder. 5. Wash bottle, multichannel pipette, or automatic microplate washer. 6. Horizontal orbital microplate shaker capable of maintaining 500±50 rpm. 7. Tubes for diluting standards and samples. Sample Collection and Storage: 1. Cell Culture Supernatant: Centrifuge at 1000×g for 15 min to remove particles. Assay immediately or aliquot samples and store at ≤-20°C. Avoid repeated freeze-thaw cycles. (Due to matrix effect, pre-experiment is recommended to determine dilution factor.) 2. Serum: Use serum separator tubes, allow samples to clot at

room temperature for 30 min, then centrifuge at 1000×g for 15 min. Remove serum immediately and assay or aliquot samples and store at ≤-20°C. Avoid repeated freeze-thaw cycles. (Due to matrix effect, serum samples are recommended to be diluted 2-fold. For example: 50 µL sample + 50 µL 1× diluent.) 3. Plasma: Collect plasma using EDTA or heparin as anticoagulant. Centrifuge at 1000×g for 15 min within 30 min after collection. Assay immediately or aliquot samples and store at ≤-20°C. Avoid repeated freeze-thaw cycles. (Due to matrix effect, plasma samples are recommended to be diluted 2-fold. For example: 50 µL sample + 50 µL 1× diluent.) Note: Citrate anticoagulant plasma has not been validated for this assay; verify feasibility when using. Hemolyzed samples are not suitable for this assay. 4. Tissue Homogenate: Rinse tissue with pre-cooled PBS (0.01 M, pH=7.4) to remove residual blood (lysed red blood cells in homogenate will affect detection results). Weigh and mince the tissue. Add corresponding volume of PBS (generally 1:9 weight/volume ratio, e.g., 1 g tissue sample corresponds to 9 mL PBS; specific volume can be adjusted according to experimental needs and recorded. Protease inhibitors are recommended to be added to PBS). Add to glass homogenizer and grind thoroughly on ice or use homogenizer. For further lysis of tissue cells, sonication or repeated freeze-thaw can be performed. Finally, centrifuge homogenate at 5000×g for 5-10 min and collect supernatant for detection. 5. Cell Lysate: For adherent cells, gently wash with pre-cooled PBS, then digest with trypsin, collect cells by centrifugation at 1000×g for 5 min; for suspension cells, centrifuge directly to collect. Wash collected cells 3 times with pre-cooled PBS. Resuspend every 1×10^6 cells in 150-200 µL PBS (protease inhibitors are recommended to be added to PBS; if content is very low, PBS volume can be appropriately reduced). Lyse cells by repeated freeze-thaw or sonication. Centrifuge extract at 2-8°C, 1500×g for 10 min and collect supernatant for detection. 6. Other Sample Types: Centrifuge at 1000×g for 20 min and collect supernatant for detection. 7. Sample Appearance: Samples should be clear and transparent; suspended matter should be removed by centrifugation. 8. Sample Storage: Samples can be stored at 4°C if tested within 1 week; if not tested promptly, aliquot according

to single-use amount and freeze at -20°C (test within 1 month) or -80°C (test within 6 months). Avoid repeated freeze-thaw. Hemolyzed specimens will affect final detection results, so hemolyzed specimens are not suitable for this assay. Reagent Preparation: 1. Equilibrate all reagents to room temperature for approximately 30 min before use. 2. Wash Buffer/Diluent Preparation: If crystals are present in 20× concentrated wash buffer/diluent, heat at 37°C until all crystals are dissolved. Dilute 1:20 with distilled water (e.g., 1 mL concentrated wash buffer + 19 mL distilled water). 3. Standard Preparation: Remove standard from kit. Prepare 7 tubes. First, take a certain amount from the 960 ng/mL standard stock and dilute with 1× diluent to 40 ng/mL (e.g., 50 µL standard stock + 950 µL 1× diluent to prepare 1000 µL of 40 ng/mL standard solution). Then add 500 µL of 1× diluent to 6 separate tubes. Perform 2-fold serial dilution of the 40 ng/mL standard in these 6 tubes to prepare 6 gradients, totaling 7 concentrations of standards: 40 ng/mL, 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL. Transfer 500 µL from the highest concentration to the next tube, mix gently by pipetting, and continue serial dilution. Use 1× diluent as the zero standard (0 ng/mL). 4. Biotinylated Antibody Working Solution: 10 min before use, centrifuge 100× biotinylated antibody at 1000×g for 1 min, then dilute with 1× diluent to 1× biotinylated antibody working solution. Prepare daily use amount daily. 5. SA-HRP Working Solution: 10 min before use, centrifuge 100× SA-HRP solution at 1000×g for 1 min, then dilute with 1× diluent to 1× SA-HRP working solution. Prepare according to required amount. Note: If the CYP2C9 concentration in samples is higher than the highest standard value, select appropriate dilution factor according to actual situation. Assay Procedure (All standards and samples are recommended to be tested in duplicate): 1. Plate Preparation: Determine the number of wells needed for the assay. Remove unused strips from the plate and return to the foil pouch with desiccant. 2. Sample Incubation: Add 100 µL of different concentrations of standards and pre-treated samples to each well (samples are recommended to be diluted at least 1-fold with universal diluent to reduce matrix effect). Cover with plate sealer and incubate at 37°C protected from light for 1 h. After incubation, add 300 µL 1× wash

	buffer to each well, gently shake for 30 s, pour out and pat dry on paper. Wash in this manner 3 times. 3. Antibody Incubation: Add 100 µL biotinylated antibody working solution to each well, mix gently, cover with plate sealer, and incubate at 37°C protected from light for 1 h. After incubation, wash 4 times using the same method as in step 2. 4. Enzyme Incubation: Add 100 µL 1× SA-HRP working solution to each well, cover with plate sealer, and incubate at 37°C protected from light for 30 min. Wash 4 times and pat dry. 5. Substrate Color Development: First add 50 µL chromogen A to each well, then add 50 µL chromogen B, mix gently, cover with plate sealer, and incubate at 37°C protected from light for 15 min. 6. Stop Reaction: After color development, add 50 µL stop solution to each well, mix gently, and measure absorbance at 450 nm using a pre-warmed microplate reader within 5 min.
Unit definition	Calculate the average OD value of standards and samples in duplicate wells and subtract the blank well OD value as the corrected value. Plot a standard curve using four-parameter logistic function with concentration as the x-axis and OD value as the y-axis (exclude blank group values when plotting). Alternatively, use computer software capable of generating four-parameter logistic (4-P) curve fitting to create the standard curve. If sample OD value exceeds the upper limit of the standard curve, dilute appropriately and re-measure, multiplying the calculated sample concentration by the corresponding dilution factor.
Storage Buffer	20× Concentrated Diluent: Dilute 1:20 with distilled water before use. If crystals are present, heat at 37°C until dissolved. 20× Concentrated Wash Buffer: Dilute 1:20 with distilled water before use. If crystals are present, heat at 37°C until dissolved.
Usage	For research use only. Not for use in diagnostic procedures. This kit is designed for the quantitative detection of human cytochrome P450 CYP2C9 in cell culture supernatant, serum, and plasma. Detection range: 0.625–40 ng/mL.

GENE INFORMATION

Gene Name	CYP2C9
Official Symbol	CYP2C9

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