Rat Glycated Hemoglobin A1c (HbA1c) ELISA Kit

Cat. No. KITG-031 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	Glycated Hemoglobin A1c (HbA1c) is a form of glycated protein. Most monosaccharides, including glucose, galactose, and fructose, when present in blood, spontaneously (i.e., non-enzymatically) bind to proteins. The formation of sugar-protein linkages indicates the presence of excess sugar in the blood, usually indicating diabetes. It is a measure of beta-N-1-deoxyfructosyl components in plasma proteins. HbA1c accounts for the glycated form of plasma proteins. HbA1c is caused by glycosylation at the N-terminus of protein amino acids, which reflects plasma glucose concentration. HbA1c concentration is mainly affected by protein and plasma glucose concentrations. However, studies have shown that factors such as race, age, diet, drugs, and concomitant diseases may also alter HbA1c levels.
Species	Rat
Description	This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In microplates pre-coated with rat glycated hemoglobin A1c (HbA1c) capture antibody, samples, standards, biotin-labeled detection antibody, and HRP enzyme conjugate are added sequentially. After incubation and washing, TMB substrate is used for color development. TMB turns blue under the catalysis of peroxidase (HRP) and turns into the final yellow color under the action of acid. The intensity of the color is positively correlated with the amount of rat glycated hemoglobin A1c (HbA1c) in the sample. The absorbance (OD value) is measured at 450 nm wavelength using a microplate reader, and sample concentration is calculated based on the standard curve.

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Form	Colorimetric ELISA Kit (96-well plate)
Applications	Quantitative detection of Rat Glycated Hemoglobin A1c (HbA1c) in serum, plasma, red blood cell lysate, and other biological samples. For research use only. Not for use in diagnostic procedures.
Notes	Kit Components: Pre-coated Assay Plate: 5×8 wells×12 strips (5×96T) / 8 wells×12 strips (96T) / 8 wells×6 strips (48T) Standard: 10 vials (5×96T) / 2 vials (96T) / 1 vial (48T) — Dilute according to manual Universal Diluent: 10×20 mL (5×96T) / 2×20 mL (96T) / 1×20 mL (48T) Biotin-antibody (100×): 5×120 µL (5×96T) / 120 µL (96T) / 60 µL (48T) — Dilute according to manual Streptavidin-HRP (100×): 5×120 µL (5×96T) / 120 µL (96T) / 60 µL (48T) — Dilute according to manual Wash Buffer (20×): 10×10 mL (5×96T) / 2×10 mL (96T) / 1×10 mL (48T) — Dilute according to manual TMB Substrate: 5×10 mL (5×96T) / 10 mL (96T) / 5 mL (48T) Stop Solution: 5×6 mL (5×96T) / 6 mL (96T) / 3 mL (48T) Plate Sealer: 20 pieces (5×96T) / 4 pieces (96T) / 4 pieces (48T) Instruction Manual: 1 copy (5×96T) / 1 copy (96T) / 1 copy (48T) Sample Processing and Requirements: 1. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. It is recommended to estimate the concentration of the analyte in the sample through relevant literature before the experiment, and determine the actual concentration of the sample through a pilot experiment. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample accordingly. 2. If the sample to be tested is not among the sample types listed in the manual, it is recommended to perform a pilot experiment to verify its detection feasibility. 3. Serum: Collect whole blood in a serum separator tube and allow to clot at room temperature for 2 hours or overnight at 2-8°C. Centrifuge at 1000×g for 20 minutes and collect the supernatant. Alternatively, store the supernatant at -20°C or -80°C, but avoid repeated freeze-thaw cycles. (For samples related to hemolytic diseases in vivo or destructive hemolysis in vitro, the target is only generated within red blood cells.) 4. Plasma: Collect samples using EDTA or heparin as an anticoagulant. Centrifuge at 2-8°C, 1000×g for 15 minutes within 30 minutes after collection, and coll

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ect the supernatant for detection. Alternatively, store the supernatant at -20°C or -80° C, but avoid repeated freeze-thaw cycles. (For samples related to hemolytic diseases in vivo or destructive hemolysis in vitro, the target is only generated within red blood c ells.) 5. Red Blood Cell Lysate: Collect a certain volume of anticoagulated blood (e.g., 1 mL), centrifuge at 4°C, 10000 rpm for 10 min, and collect the red blood cells. Discar d the supernatant, add pre-cooled pure water to resuspend to the original volume (e. g., 1 mL), freeze at -20°C for 30-60 minutes, and repeat freeze-thaw for 3 rounds to d estroy the cell membrane. Then centrifuge the cell suspension at 4°C, 10000 rpm for 10 minutes, collect the supernatant, and detect immediately; or aliquot and store at -2 0°C, but avoid repeated freeze-thaw cycles. Thawed samples should be centrifuged a gain before detection. 6. Sample Appearance: Samples should be clear and transpar ent. Suspended matter should be removed by centrifugation. 7. Sample Storage: Sam ples collected for detection within 1 week can be stored at 4°C. If detection cannot be performed in time, please aliquot according to single-use volume and freeze at -20°C (for detection within 1 month) or -80°C (for detection within 6 months). Avoid repeated freeze-thaw cycles. Sample hemolysis will affect the final detection results, so hemoly zed samples are not suitable for this assay. Sample Dilution Protocol: Please estimat e the concentration range of the sample in advance. If your sample needs dilution, ref er to the following dilution protocol: Dilute 100-fold: One-step dilution. Take 5 µL of sa mple into 495 µL of universal diluent for 100-fold dilution. Dilute 1000-fold: Two-step d ilution. Take 5 µL of sample into 95 µL of universal diluent for 20-fold dilution, then tak e 5 µL of the 20-fold diluted sample into 245 µL of universal diluent for 50-fold dilutio n, total 1000-fold dilution. Dilute 100000-fold: Three-step dilution. Take 5 µL of sampl e into 195 µL of universal diluent for 40-fold dilution, then take 5 µL of the 40-fold dilut ed sample into 245 µL of universal diluent for 50-fold dilution, and finally take 5 µL of t he 2000-fold diluted sample into 245 µL of universal diluent for 50-fold dilution, total 1 00000-fold dilution. At each dilution step, the volume taken should not be less than 3 µL, and the dilution factor should not exceed 100-fold. Each dilution step must be mix ed thoroughly to avoid errors. Reagent Preparation: 1. Please remove the kit from the

refrigerator 10 minutes in advance and equilibrate to room temperature. 2. Standard g radient working solution preparation: Add 1 mL of universal diluent to the lyophilized s tandard, let it stand for 15 minutes to completely dissolve, then gently mix (concentrati on: 2000 ng/mL). Then prepare dilutions according to the following concentrations: 20 00 ng/mL, 1000 ng/mL, 500 ng/mL, 250 ng/mL, 125 ng/mL, 62.5 ng/mL, 31.25 ng/mL, 0. Serial dilution method: Take 7 EP tubes, add 500 µL of universal diluent to each tu be. Take 500 µL from the 2000 ng/mL standard working solution into the first EP tube and mix well to prepare 1000 ng/mL standard working solution. Continue taking 500 µ L from each tube to the next tube and mix well. The last tube serves directly as the bl ank well; no need to take liquid from the second-to-last tube. Standard concentration s: 2000➔1000➔500➔250➔125➔62.5➔31.25➔0 (ng/mL). 3. Biotin-labeled detection antibo dy working solution preparation: 15 minutes before use, centrifuge the 100× concentrate d biotin-labeled detection antibody at 1000×g for 1 minute, and dilute the 100× concentra ted biotin-labeled detection antibody to 1× working concentration with universal diluent (e.g., 10 µL concentrate + 990 µL universal diluent). Prepare fresh before use. 4. Enzy me conjugate working solution preparation: 15 minutes before use, centrifuge the 10 0× concentrated enzyme conjugate at 1000×g for 1 minute, and dilute the 100× conce ntrated enzyme conjugate to 1× working concentration with universal diluent (e.g., 10 µL concentrate + 990 µL universal diluent). Prepare fresh before use. 5. 1× Wash Buf fer preparation: Take 10 mL of 20× wash buffer into 190 mL of distilled water. (Crystal s may form in the concentrated wash buffer taken from the refrigerator; this is normal. Place at room temperature until crystals are completely dissolved before use.) Assay Procedure: 1. Remove the required number of strips from the foil pouch after equilibra ting at room temperature for 10 minutes. Seal the remaining strips in a self-sealing ba g and store at 4°C. 2. Sample addition: Add 100 µL of sample or different concentrati ons of standards per well. Add 100 µL of universal diluent to blank wells. Cover with p late seal and incubate at 37°C for 60 minutes. (Recommendation: Dilute the samples to be tested with universal diluent at least 1-fold before adding to the enzyme plate for testing, thereby reducing the influence of matrix effects on test results. When calculati

ng sample concentration, multiply by the corresponding dilution factor. It is recommended to set up duplicate wells for all samples and standards during detection.) 3. Add biotin-labeled detection antibody: Remove the enzyme plate, discard the liquid, do not wash. Add 100 µL of biotin-labeled detection antibody working solution directly to each well. Cover with plate seal and incubate at 37°C for 60 minutes. 4. Wash plate: Discard the liquid. Add 300 µL of 1× wash buffer to each well, let stand for 1 minute, shake off the wash buffer, and pat dry on absorbent paper. Repeat washing 3 times (or use a plate washer). 5. Add enzyme conjugate working solution: Add 100 µL of enzyme conjugate working solution to each well. Cover with plate seal and incubate at 37°C for 30 minutes. 6. Wash plate: Discard the liquid and wash 5 times according to the washing method in step 4. 7. Add substrate: Add 90 µL of substrate (TMB) to each well. Cover with plate seal and incubate at 37°C in the dark for 15 minutes. 8. Add stop solution: Remove the enzyme plate and add 50 µL of stop solution directly to each well. Immediately measure the OD value of each well at 450 nm. Data Analysis: 1. Calculate the average OD value of standard and sample duplicate wells and subtract the OD value of the blank well as the corrected value. Plot a standard curve with concentration on the x-axis and OD value on the y-axis using a four-parameter logistic function on log-log coordinate paper. 2. If the sample OD value exceeds the upper limit of the standard curve, dilute appropriately and retest, multiplying by the corresponding dilution factor when calculating sample concentration. Kit Performance: 1. Repeatability: Intra-assay coefficient of variation is less than 10%, inter-assay coefficient of variation is less than 10%. 2. Recovery: Three different concentration levels of rat HbA1c were added to selected healthy rat red blood cell lysate, and recovery was calculated. Sample Type: Red Blood Cell Lysate (n=8) Range: 84-101% Average Recovery: 96% 3. Linearity Dilution: High concentration rat HbA1c was added to selected healthy rat red blood cell lysate, and dilutions were performed within the standard curve dynamic range to assess linearity. Dilution Ratio 1:2: Range 84-99%, Average 92% Dilution Ratio 1:4: Range 82-103%, Average 93% Precautions: 1. Strictly follow the specified time and temperature for incubation to ensure accurate results. All reagents must be brought to roo

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	m temperature (20-25°C) before use. Reagents should be refrigerated immediately after use. 2. Incorrect plate washing may lead to inaccurate results. Ensure that the liquid in the wells is removed as much as possible before adding the substrate. Do not allow the microplate to dry out for too long during the entire process. 3. Clean any residual liquid on the bottom of the plate and fingerprints, otherwise they will affect the OD value. 4. The substrate color solution should be colorless. Do not use substrate solution that has turned blue. 5. Avoid cross-contamination between reagents and samples to prevent erroneous results. 6. Avoid direct exposure to strong light during storage and incubation. 7. Do not allow any reactive reagents to come into contact with bleach solutions or strong gases emitted by bleach solutions. Any bleaching agent will destroy the biological activity of the reagents in the kit. 8. Do not use expired products. Components from different catalog numbers and batch numbers must not be mixed. 9. Recombinant proteins from sources outside the kit may not be recognized by the antibodies in this kit. 10. If potentially infectious, all samples should be handled and managed properly, and samples and detection devices should be processed according to established procedures. Materials Required but Not Supplied: 1. Microplate reader (450 nm) 2. High-precision pipettes and tips: 0.5-10 µL, 5-50 µL, 20-200 µL, 200-1000 µL 3. 37°C incubator 4. Distilled or deionized water
Storage	Store at 2-8°C, protected from light and moisture. Valid for 6 months.
Shipping	Shipped at ambient temperature.
EC	1× Wash Buffer (dilute 20× with distilled water 1:20), Universal Diluent
Storage Buffer	1× Wash Buffer (dilute 20× with distilled water 1:20), Universal Diluent
Labeling Efficiency	For research use only. Not for use in diagnostic procedures.

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