

Mouse Glycated Hemoglobin (HbA1c) ELISA Kit

Catalog No.: abx156705

Size: 96T

Range: 12.35 µg/ml - 1000 µg/ml

Sensitivity: < 4.55 µg/ml

Storage: Store standard, detection reagent A, detection reagent B and the 96-well plate at -20°C, and the rest of the kit components at 4°C.

Application: For quantitative detection of HbA1c in Mouse Plasma, Cell Lysates and other biological fluids.

Introduction: Glycated hemoglobin (hemoglobin A1c, HbA1c, A1C, or less commonly HgbA1c, haemoglobin A1c, HbA1c, Hb1c, etc.) is a form of hemoglobin that is covalently bound to glucose. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. It is measured primarily to identify the three-month average plasma glucose concentration and thus can be used as a diagnostic test for diabetes and as assessment test for glycemic control in people with diabetes. The test is limited to a three-month average because the lifespan of a red blood cell is four months (120 days). However, since red blood cells do not all undergo lysis at the same time, HbA1C is taken as a limited measure of three months. HbA1c is a measure of the beta-N-1-deoxy fructosyl component of hemoglobin.

Principle of the Assay

This kit is based on a competitive binding enzyme-linked immuno-sorbent assay technology. An antibody specific to HbA1c is pre-coated onto the 96 well plate. A competitive inhibition reaction is launched between biotin labeled HbA1c and unlabeled HbA1c with the pre-coated antibody specific to HbA1c. After washing away the unbound conjugates, avidin conjugated to Horseradish Peroxidase is added to each microplate well and incubated. After TMB substrate solution is added only wells that contain HbA1c will produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is inverse proportional to the HbA1c amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of HbA1c can be calculated.

Kit components

1. One pre-coated 96-well microplate (12 × 8 well strips)
2. Standard: 2 tubes
3. Standard Diluent Buffer: 20 ml
4. Wash Buffer (30X): 20 ml. Dilution: 1:30
5. Diluent A: 12 ml
6. Diluent B: 12 ml
7. Stop solution: 6 ml
8. TMB substrate : 9 ml
9. Detection Reagent A (100X): 120 µl
10. Detection Reagent B (100X): 120 µl
11. Plate sealer: 4

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Multi and single channel pipettes and sterile pipette tips
4. Squir bottle or automated microplate washer
5. ELISA shaker
6. Tubes to prepare standard or sample dilutions
7. Deionized or distilled water
8. Absorbent filter papers
9. 100 ml and 1 liter graduated cylinders

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Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, analyze immediately or store at 4°C for up to 5 days. Otherwise, store at -20°C for up to one month or -80°C for up to two months to avoid loss of bioactivity. Avoid multiple freeze-thaw cycles.

- **Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Cell lysates:** Detach adherent cells with trypsin and collect by centrifugation and remove the supernatant. Wash the cells three times in ice-cold PBS and re-suspend cells in PBS. Lyse the cells by ultra-sonification 4 times or freeze at -20°C and thaw to room temperature 3 times. Centrifuge at 1500 × g for 10 min at 2-8°C to remove cellular debris. Collect the supernatant and assay immediately.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

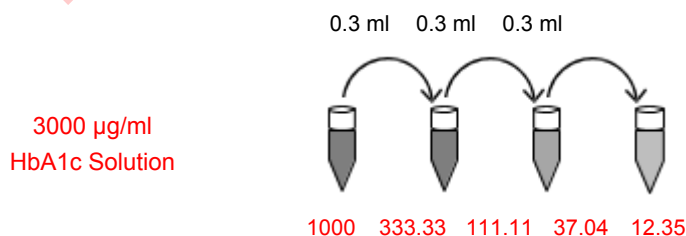
- » Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.
- » Samples must be diluted so that the expected concentration falls within the kit's range. Sample should be diluted in 0.01 mol/L PBS (PH=7.0-7.2). Serum/plasma samples require approximately a 10 fold dilution.
- » If the sample are not indicated in the manual's applications, a preliminary experiment to determine the validity of the kit will be necessary.
- » Fresh sample or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.
- » Always use non-pyrogenic, endotoxin-free tubes for blood collection.

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

3. Standard

Bring samples and all kit components to room temperature. Prepare the Standard with 1.0 ml of Standard Diluent buffer (kept for 10 min at room temperature) to make the 3000 µg/ml Standard Solution. Allow the reconstituted standard to sit for 10 minutes with gentle agitation prior to carrying out the serial dilutions; avoiding foaming or bubbles. Further dilute by a factor of 3 to create the highest standard (1000 µg/ml). Label 3 tubes with 333.33 µg/ml, 111.11 µg/ml, 37.04 µg/ml, 12.35 µg/ml. Aliquot 0.6 ml of the Standard diluent buffer into each tube. Add 0.3 ml of 1000 µg/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.3 ml from 1st tube to 2nd tube, mix thoroughly, and so on.



4. Detection Reagent A and B Preparation

Centrifuge Detection Reagent A and B briefly before use. Detection Reagent A and B should be diluted 100-fold with the Diluent A and B respectively and mixed thoroughly. They are sticky solutions, therefore pipette with a slow, smooth action to reduce volume

errors. The solution should be prepared no more than 2 hours prior to the experiment. Please discard after use.

B. Assay Procedure

Equilibrate the kit components and samples to room temperature before use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate or triplicate. Add the solution at the bottom of each well without touching the side walls.
2. Add 50 µl of the diluted standards into the standard wells. Aliquot 50 µl Standard Diluent Buffer to the control (zero) well.
3. Add 50 µl of appropriately diluted sample into the test sample wells. Add the solution at the bottom of each well without touching the side wall. Shake the plate mildly to mix thoroughly.
4. Immediately aliquot 50 µl of Detection Reagent A working solution (if it appears cloudy mix gently until the solution is uniform) to each well. Shake the plate gently to mix thoroughly (a microplate shaker is recommended).
5. Seal the plate with a cover and incubate for 1 h at 37°C.
6. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or autowasher (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
7. Aliquot 100 µl of Detection Reagent B working solution into each well, seal and incubate at 37°C for 30 min.
8. Repeat the aspiration/wash process 5 times as explained in step 6.
9. Aliquot 90 µl of TMB Substrate into each well. Seal the plate with a cover and incubate at 37°C for 10-20 min. Avoid exposure to light. The incubation time is for reference use only, the optimal time should be determined by end user. Do not exceed 30 min.
10. Add 50 µl of Stop solution into each well to stop the enzyme reaction. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
11. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

This assay is competitive, therefore there is an inverse correlation between HbA1c concentration in the sample and the absorbance measured. Create a graph with the log of the standard concentration (y-axis) and average absorbance measured (x-axis). Apply a best fit trendline through the standard points. The HbA1c concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor by the interpolated concentration of the sample to obtain the concentration before dilution.

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C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard dilutions within 15 min of use and discard any unused working standards. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
4. It is recommended measuring each standard and sample in duplicate or triplicate.
5. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells, avoid letting the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. Do not reuse pipette tips and tubes to avoid cross contamination.
9. Do not vortex the standard during reconstitution, as this will destabilize the protein. Once your standard has been reconstituted, it should be used right away. We do not recommend reusing the reconstituted standard.
10. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of HbA1c were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of HbA1c were tested on 3 different plates, 8 replicates in each plate.

$$CV (\%) = (\text{Standard Deviation} / \text{mean}) \times 100$$

Intra-Assay: CV<10%

Inter-Assay: CV<12%

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E. Typical Data & Standard Curve

Typical Standard Curve Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Concentration $\mu\text{g/ml}$	12.35	37.04	111.11	333.33	1000
OD450	1.254	0.804	0.499	0.285	0.132

