Human Glycated Hemoglobin (HbA1c) CLIA Kit

Catalog No.: abx490036

Size: 96T

Range: 5.86 µg/ml - 1500 µg/ml

Sensitivity: < 2.44 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 Human Serum, Plasma and cell lysates.

Introduction: Glycated Hemoglobin (HbA1c) is a form of hemoglobin that has been covalently bound to glucose without the aid of enzymes; glycosylated hemoglobin is preferentially used to describe hemoglobin-glucose compounds that were made using enzymes. Plasma levels of HbA1c naturally rise when blood sugar concentrations increase, however elevated HbA1c concentrations are indicative of diabetes mellitus, and are commonly used as a biomarker of this disease.

Principle of the Assay

This kit is based on competitive chemiluminescent immunoassay technology. HbA1c antibody is pre-coated onto a 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 the mixture of substrate A and B is added only wells that contain biotin labeled HbA1c will produce chemiluminescence. The intensity of the emitted light is inversely proportional to the amount of HbA1c in the sample or standard.

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. Detection Reagent A (100X): 120 µl
6. Detection Reagent B (100X): 120 µl
7. Diluent A: 12 ml
8. Diluent B: 12 ml
9. Substrate A: 10 ml
10. Substrate B: 2 ml
11. Plate sealer: 4

Material Required But Not Provided

1. 37°C incubator
2. Luminometer capable of reading 96-well microplates (lag time 30.0 secs and read time 1.0 sec/well)
3. Multi and single channel pipettes and sterile pipette tips
4. Squirt bottle or automated microplate washer
5. Deionized or distilled water
6. Tubes to prepare standard or sample dilutions
7. Absorbent filter papers
8. 100 ml and 1 L volume graduated cylinders

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.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 1000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **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.
- **Erythrocyte lysates:** Centrifuge whole blood at approximately 1000 × g for 20 minutes. Wash pellet three times in cold PBS (0.02 mol/L pH 7.0-7.2). Resuspend cells in PBS and freeze at -20°C, then thaw at room temperature. Repeat this 3 times. Centrifuge at 5000 × g for 10 minutes at 2-8°C to remove cellular debris. Collect the supernatant and assay 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).

» 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. For better detection, it is highly recommended to use serum instead of plasma.

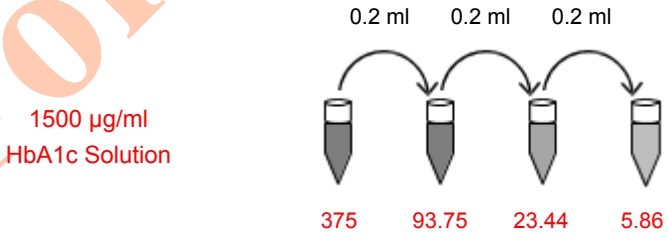
» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

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 ml of Standard Diluent buffer (kept for 10 min at room temperature) to make the 1500 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. Label 4 tubes with 375 µg/ml, 93.75 µg/ml, 23.44 µg/ml, 5.86 µg/ml. Aliquot 0.6 ml of the Standard diluent buffer into each tube. Add 0.2 ml of 1500 µg/ml standard solution into the 1st tube and mix thoroughly. Transfer 0.2 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 and mixed thoroughly. They are sticky solutions, therefore pipette with a slow, smooth action to reduce volume errors. Please discard after use.

5. Substrate working solution Preparation

Substrate A and B should be mixed with a ratio of 99:1 respectively and mixed thoroughly. For example, prepare 1 ml of substrate working solution by mixing 0.99 ml of Substrate A and 0.01 ml of Substrate B.

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 100 µl of Substrate working solution into each well. Seal the plate with a cover and incubate at 37°C for 10 min. Avoid exposure to light. The incubation time is for reference only, the optimal time should be determined by the end user.
10. Measure the chemiluminescence signal in a microplate luminometer immediately.

This assay is competitive, therefore there is an inverse correlation between HbA1c concentration in the sample and the RLUs (Relative Light Units) 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.

C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. Wash buffer may crystallize and separate. If this happens warm to room temperature and mix gently until the crystals are completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components. Prepare the Standard solutions within 15 min of starting the experiment. Please use the diluted Standard for a single assay procedure and discard after use. 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.
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 Substrate solution is easily contaminated; work under sterile conditions when handling the substrate solution. The Substrate A and B 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 (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Sample Recovery (after spiking)

Sample Type	Range	Average Recovery
Serum (n=5)	83-95%	89%
EDTA Plasma (n=5)	90-103%	96%
Heparin Plasma (n=5)	97-105%	101%

Linearity

Sample Type	1:2	1:4	1:8	1:16
Serum (n=5)	93-104%	85-95%	89-101%	87-96%
EDTA Plasma (n=5)	84-96%	89-99%	95-105%	92-101%
Heparin Plasma (n=5)	86-96%	94-106%	84-94%	94-107%