NEPHSTAR®
Haemoglobin A1c (HbA1c）Kit

Catalog No. DK071

1. Intended Use

This product is used on NEPHSTAR® protein analysis system for quantitative determination of human Haemoglobin A1c (HbA1c) percentage to total Haemoglobin in whole blood as an aid in diagnosis of diabetic mellitus.

2. Summary

Diabetes Mellitus is a chronic disease characterized by a hyperglycemia. The consequences are metabolism disorders of carbohydrates, lipids and proteins. The risk of complications associated with diabetes, including nephropathy, retinopathy and cardiovascular diseases, increases in patients with poor metabolic control. In the diabetic patients, where blood glucose levels are elevated, HbA1c is formed as a consequence of the non-enzymatic glycation of the N-terminus of the β -chain of haemoglobin molecule. The level of HbA1c is proportional to the level of glucose in the blood and has been widely accepted as an indicator of the mean daily blood glucose concentration over the preceding 6-8 weeks. It is therefore, a long-term indicator of diabetic control, whereas, the measurement of blood glucose is only a short-term.

3. Test Principle

The HbA1c kit is a quantitative nephelometric test directly for the percentage of glycated hemoglobin (HbA1c) to total haemoglobin (HbT). Total haemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When mouse anti-human HbA1c monoclonal antibody is added, latex-HbA1c-mouse anti-human HbA1c antibody complex is formed. Agglutination is formed when goat anti-mouse igG polyconal antibody interacts with the monoclonal antibody. The amount of agglutination is measured by nephelometry. The HbA1c percentage value is obtained from a calibration curve.

4. Kit Components

Code	Name	Volume/quantity
DL071	NEPHSTAR HbA1c R1 (Latex)	3×2.5 mL
DA071	NEPHSTAR HbA1c R2a	1×1.0 mL
DB071	NEPHSTAR HbA1c R2b	1×2.5 mL
DH071	NEPHSTAR Haemolysis Solution	1×25 mL
DC071	NEPHSTAR HbA1c Card	1
	Manual	1

5. Materials required but not supplied

5.1 NEPHSTAR Protein analysis system（NS100）
5.2 NEPHSTAR Accessory pack（DK110）
5.3 Pipette 5-50μL
5.4 Electronic pipette (YB201)
5.5 Pipette 100-1000μL
5.6 Equipment for collection of samples
5.7 NEPHSTAR HbA1c Control（Code: DM071，1×0.5mL）

6. Storage and Stability

The unopened reagent kit should be stored under 2-8℃ avoiding direct sunlight and can be used until the expiry date labeled on the kit. **Do not freeze!** Once opened the HbA1c latex (R1) is stable for 60 days at 2-8℃, the antibody (R2a and R2b) is stable for 30 days at 2-8℃.

7. Reagent preparation

7.1 HbA1c R1,R2a,R2b： Ready to use.

7.2 HbA1c Control: Open the vial and accurately add 0.5 mL haemolysis solution into the vial to dissolve the contents. Gently mix for 10 minutes, or until all material has dissolved. The reconstituted control is ready to use as **whole blood sample**. The reconstituted control is stable for **30** days if stored tightly closed at 2-8℃. The reconstituted control is stable for 6 months if stored tightly closed at -20℃

8. Sample collection and preparation

Haemolysis of samples: Dispense 1mL haemolysis solution into a test tube, add 10μL of whole blood sample into the tube, then mix. Allow the mixture to stand for 5 minutes or until complete lysis is evident. The blood sample can be fresh EDTA venous blood or freshly collected on finger tip. The haemolysate is stable for 48 hours at 2-8℃.

9. Interferences:

No interferences for ascorbic acid(50mg/dL), Triglyceride (2000mg/dL), Bilirubin (40mg/dL), carbamylated Hb (7.5mmol/L) and acetylated Hb (5 mmol/L).

10. Test Procedure

Summary: Reagent volumes added to the cuvette

Reagent	Volume
Haemolysate	10μL
HbA1c R1	300μL
Addition after 3 minutes	
HbA1c R2b	100μL
HbA1c R2a	**40**μL

All reagents should be equilibrated fully to room temperature before use!

10.1 Switch NEPHSTAR on.
10.2 **Enter chemistry number**. Enter chemistry number of HbA1c kit (HbA1c=71). If HbA1c assay has never been performed on the instrument before, please swipe card when “please swipe card” is displayed.
10.3 The assay name and lot of reagent are displayed. Check carefully, press ENTER if the lot number is identical to the last three or four digits of the number printed on the card or kit label, otherwise swipe card to update the curve data stored in NEPHSTAR.
10.4 **Enter sample ID.** Press number keys to enter the sample ID; or press ENTER to accept the currently displayed sample ID.
10.5 Enter sample dilution: 1. Accept the default sample dilution by pressing ENTER. For HbA1c assay the haemolysate should not be diluted.
10.6 Prepare one cuvette for each sample to be assayed. Place a stirring bar in the cuvette using the forceps

supplied with NEPHSTAR. Add 10μL sample haemolysate on the bottom of the cuvette.

10.7 **Place cuvette in chamber.** Place the cuvette in chamber, press it lightly until it contacts the bottom of the chamber. The cuvette will be detected.

10.8 **Add reagent**. Add 300μL HbA1c R1 to the cuvette. NEPHSTAR will sense the addition of R1, the stirring bar will stir and time will be counted down automatically for 3 minutes. You can also count the time with other method and choose to skip this step by pressing SKIP.

10.9 **Add reagent.** When 3-minute time count is finished, NEPHSTAR will beep and indicate addition of reagent. Add 100μL HbA1c R2b and 40μL R2a simultaneously into the cuvette using the electronic pipette (Cat. No: YB201) and **immediately press ENTER** to start the assay. At the end of the assay the result will be displayed and printed automatically.

- **Note: If the assay begins before reagent R2 is added, just pull the cuvette out of the chamber and place it back in the chamber again and turn to step 10.8.**

10.10 On completion of the assay remove the cuvette, press ENTER to perform the next assay. The step will be turned to 10.7. Sample ID will increase sequentially. For alteration of the ID press BACK twice and tip in the right number.

10.11 If NEPHSTAR indicates result is higher or lower than measurement range and exact result is expected, you will have to reassay the sample with other method. Changing the dilution of the sample will result in misleading values.

10.12 On completion of all assays of the same chemistry press ESC and return to step 10.2. Enter new chemistry number and begin another chemistry assay.

11. Quality Control

In accord with good laboratory practice, users are suggested to run control with every batch of samples. Results of control should fall in the validity range labeled on the control vial.

Note: HbA1c control is not standard component of the kit.

12. Sensitivity and measuring range

The sensitivity limit is 3% and the upper limit is 15%.

13. Antigen Excess

Sample HbA1c percentage concentration of less than 40% will not result in antigen excess. But such high HbA1c concentration of patient sample will not happen.

14. Reference Range

14.1 According to WHO，normal range of HbA1c percentage of healthy adult is：3.8 – 5.8%. For diabetic patients, less then 7% of HbA1c percentage is acceptable. We recommend local reference ranges are produced.

14.2 Diagnosis and treatment can not only depend on determination of HbA1c. The clinical symptoms and other laboratory findings of respective patients should be taken into consideration。

15. Precision

Two analyte concentrations are assayed within several days using this kit of the same lot on NEPHSTAR. 20 repeat assays are performed for each concentration. The average coefficient variations (CV) for each concentration are displayed in the following table:

HbA1c（%）	CV（%）
5	4.46
11.8	2.58

16. Correlation Study

A correlation study is performed on 20 clinical samples using this kit on NEPHSTAR and another system using HPLC method. The linear regression equation and correlation equation got as showed below demonstrate a good correlation between the two methods:

Y=0.981X – 0.10

(Y= NEPHSTAR® HbA1c，X=HPLC HbA1c)

Correlation coefficient r=0.978

17. Caution and Warning

17.1 The reagents are only for in vitro diagnostic use。

17.2 The reagents can be used only by trained personnel and good laboratory practice (GLP) and the stated procedure should be abided strictly.

17.3 All sera or blood have been tested to be HIV(1&2) antibody negative, HBsAg negative. However, the performed testing method can not assure the absolute absence of infectious agents in blood products, so please be sure to handle the blood products such as controls and antisera as potentially infectious sources.

17.4 All reagents of the kit contain sodium azide as preservative. Take caution when handling them. Ingest or contact of the reagents with skin or mucous membranes is forbidden. Wash with large amount of water and seek medical advice if contact does occur. In addition, explosive metal azides may be formed with lead or copper plumbings; when disposing the reagents be sure to flush with large amount of water to avoid buildup of azide.

17.5 All components of kit are NEPHSTAR® specific. Reagents of different lots are not interchangeable, otherwise the results can be misleading.

18. Referrences

Cohen P.M. Perspective: measurement of Circulating Glycated Protein to Monitor Intermediate – Term Changes in Glycaemic Control Eur J Clin Chem. Clin. Biochem. 1992:30 (12): 851 – 859

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