# Hemoglobin A1c (HBA1c)

## Turbidimetric Immunoassay

**Intended Use**

Biomed Diagnostics Hemoglobin A1c reagent is intended for Quantitative turbidimetric determination of HbA1c in human blood

**Background**

The glycemic control in diabetes mellitus is mainly by the determination of glucose, but also through quantitative determination of hemoglobin A1c in human blood. HbA1c is an indication for the actual glucose levels over the preceding 3 months. It was shown that HbA1c in diabetic subjects can be elevated 2-3 fold over normal and on other hand approaches normal values when they are under metabolic control.

**Assay Principle**

This method utilizes the interaction of antigen and antibody to determine the HbA1c in whole EDTA blood. HbA1c in test samples is absorbed onto the surface of latex particles, which react with Anti-HbA1c (antigen-antibody reaction) and gives agglutination. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve.

**Reagent**

Reagent1

Latex, Sodium azide (0.95 g/L)

Reagent2

Anti-human hemoglobin A1C mouse monoclonal antibody. Stabilizers.

Reagent3

Hemolysis reagent

**Materials required but not provided with the kit**

1-Standard set

HbA1c concentration is stated on the vials labels.

2-Controls

**Reagent Preparation, Storage and Stability**

Biomed HbA1c reagents are stable up to the expiry date labeled on the bottles when stored at 2 - 8 °C and contaminations are Prevented during their use. Once opened the reagents are stable for 1 month if stored tightly closed at 2 - 8 ºC after use.

**Specimen Collection and Preparation**

Fresh EDTA blood.

**Hemolysate procedure**

To determine HbA1c, a hemolysate must be prepared for each sample as follow:

1-Dispense 1 ml hemolysis reagent into a test tube.

2-Place 10 µl of well mixed whole EDTA blood (Samples, Calibrator and Controls) into the test tube and mix.

3. Allow to rest 10 minutes or until complete lysis is evident. Stability of the hemosylate: 72 hours at 2 - 8°C.

**Procedure**

| | |
|---|---|
| Wavelength | 630 nm |
| Temperature | 37 °C |
| Cuvette | 1cm light path |
| Zero adjustment | distilled water |

**Use hemolysated Sample/Calibrator/control**

| | Calibrator | Sample |
|---|---|---|
| Reagent (R1) | 200 µl | 200 µl |
| Calibrator | 5 µl | |
| Sample/control | | 5 µl |
| Mix, and incubate for 2 minutes then add | | |
| Reagent (R2) | 40 µl | 40 µl |

Mix and read absorbance (A1) after 30 sec., incubate for 3 minutes and read absorbance (A2)

**Calculation**

Generate a reference curve using HbA1c Calibrator set. Determine:

Delta absorbance of the sample and each standard as following:

Delta absorbance of sample = (A2 - A1) sample

Delta absorbance of each standard = (A2 - A1) for each Standard

Plot the calibration curve and obtain the result.

**Expected Values**

| | |
|---|---|
| Non-diabetics | < 6 % |
| Therapeutic diabetics | < 7 % |

Each laboratory should establish its own reference range.

**Linearity**

Up to 15 %

Specimens showing higher concentration should be diluted 1/5 using

Physiological saline and repeat the assay.

**Dynamic Range**

0 – 15 %

**References**

1-Bates, H.M., Lab. Mang., Vol 16 (Jan. 1978(

2-Gonen, B., and Rubenstein, A.H., Diabetologia 15, 1 (1978)

3-Trivelli, L.A., Ranney, H.M., and Lai, H.T., New eng. J. Med. 284,353 (1971)

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