**Hemoglobin A1c (HbA1c)**

**Turbidimetric Immunoassay**

**Intended Use**

Spectrum 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**

Reagent 1 (R1) *(Avoid freezing)*

- Latex.
- Sodium azide (0.95 g/L).

Reagent 2 (R2)

- Anti-human hemoglobin A1c mouse monoclonal antibody.

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

1. Standard set
2. Controls

**Reagent Preparation, Storage and Stability**

Spectrum HbA1c reagents are stable up to the expiry date labeled on the bottles when stored at 2 - 8ºC *(Avoid freezing)* and contaminations are prevented during their use. Once opened the reagents are stable for 1 month if stored tightly closed at 2 - 8 ºC and on the other hand approaches normal values when they are under metabolic control.

**Specimen Collection and Preparation**

Fresh EDTA blood.

**Hemolysate procedure**

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

1. Dispense 2 ml hemolysis reagent into a test tube.
2. Place 20 ml of well mixed whole EDTA blood (Samples, Standards and Controls) into the test tube and mix.
3. Allow to rest 5 minutes or until complete lysis is evident. Stability of the hemolysate: 72 hours at 2 - 8ºC.

**Procedure**

- Wavelength: 650 nm
- Temperature: 37 ºC
- Cuvette: 1 cm light path
- Zero adjustment: distilled water

**Calculate**

Mix and incubate for 2 minutes, then add

Reagent (R2) 75 ul

Mix and read absorbance (A1) immediately, then after 5 minutes read absorbance (A2).

**Adaptation**

Sheets for several automatic analyzers are available upon request.

**Calculation**

Generate a reference curve using HbA1c standard set. Determine D absorbance of the sample and each standard as following:

D absorbance of sample = (A2 - A1) sample
D 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 %.

**Waste Disposal**

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

SS5: dispose of this material and its container at hazardous or special waste collection point.

SS7: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. refer to special instructions/safety data sheets.

**References**


**Waste Disposal**

- Use by/Expiration Date
- Catalogue Number
- Reference Code
- Authorised Representative
- Temperature Limitation
- Use by/Expiration Date
- Temperature Limitation

**ORDERING INFORMATION**

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