

# Hemoglobin A<sub>1c</sub>

## (HbA<sub>1c</sub> direct colorimetric Test)

Cat.No	Package Size	
837 100	R1 (R1a+R1b) => 23,5 mL	R2 = 10 mL
	Lysing Reagent = 25 ml	

### GENERAL

The glyemic control in diabetes mellitus is performed mainly by the determination of Glucose, but also through quantitative determination of Hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>) in human blood : HbA<sub>1c</sub> is an indication for the actual glucose levels over the preceding 3 months. It was shown that HbA<sub>1c</sub> in diabetic subjects can be elevated 2-3 fold over normal and on the other hand approaches normal values when they are under metabolic control.

### PRINCIPLE

Hemolyzed blood is used as sample material. Through protease attack glycosylated valines are released and are subject to further enzymatic reaction with fructosyl valine oxidase (FVO) . The result is a measurable quantitative amount of hydrogen peroxide, which is determined through colorimetric reaction with a chromogen compound.

The reaction product is proportional to the amount of HbA<sub>1c</sub> and is measured as absorbance A. The HbA<sub>1c</sub> value is derived from a calibration curve.

### REAGENTS

<b>R1a:</b>	MES-Buffer (pH 7.0)	5.0 mmol/l
	Protease	≥ 3,5 kU/ml
	Triton-X 100	0.5 %l
	Redox compound	
<b>R1b:</b>	MES-Buffer (pH 6.3)	1.0 mmol/l
	Redox compound	
<b>R2:</b>	TRIS-Buffer (pH 8.0)	5.0 mmol/l
	Fructosyl Valine Oxidase	≥ 9,5 kU/l
	Peroxidase	≥ 8,5 kU/l
	Chromogen	≥ 0.7 mmol/l

### Storage

Store all reagents refrigerated at 2-8°C. Unopened reagents are stable up to the expiration date printed on the labels.

### Preparation of Reagents

#### 3-reagent-procedure:

**R1a, R1b and R2** are ready for use when the 3-reagent-procedure is applied.

*Stability after opening :*

**At least 3 months when contamination is avoided**

#### 2-reagent-procedure:

**R1:** Mix 7 parts R1a with 3 parts R1b

*Stability:*

**At least 2 weeks when contamination is avoided**

**Important Note: R1b and R2 are light sensitive !**

### Additional Reagents

**Calibration Set: Cat. No. 270 410 (2 x 0.5 ml)**

**Control Set: Cat. No. 270 510 (2 x 0.5 ml)**

### SAMPLES

Collect venous blood with EDTA.

#### Storage and Stability

*Hemoglobin A<sub>1c</sub> in whole blood with EDTA is stable for one week at 2-8 °C.<sup>5</sup>*

### To determine HbA<sub>1c</sub>, a hemolysate must be prepared from each sample:

1. Dispense 250µl of Lysing Reagent into cups or tubes and label as Controls, Patients, etc.
2. Add 20ul of well mixed (!) whole blood samples respectively of Calibrators and Controls .  
(Note: Calibrators and Controls have to be treated exactly like the patient samples!)
3. Let incubate at room temperature for minimum 10 min.

*Stability:*

**Hemolysates may be stored up to 1 day at 2-8°C**

### PRECAUTIONS

- 1.The reagent is for in vitro diagnostic use only.
- 2.All human specimens should be regarded as potentially biohazardous. Therefore, universal precautions should be used in specimen handling (gloves, lab garments, avoid aerosol production, etc.)

### ANALYTICAL PROCEDURE

***This reagent is made esp. for use on automated analyzers. Applications are available on request. Following (as an example) you find the instrument setting for the Hitachi 717 which is using the "2-reagent procedure"***

ASSAY CODE	[2-POINT]:[24]-[40]
SAMPLE VOLUME	[20] [20]
R1 VOLUME	[128] [20] [NO]
R2 VOLUME	[56] [20] [NO]
WAVELENGTH	[ 800 ] [700]
CALIBRATION	[LINEAR] [0]-[0]
STD (1) CONC-POS	[**] [1]
STD (2) CONC-POS	[**] [1]
STD (3) CONC-POS	[0] [0]
STD (4) CONC-POS	[0] [0]
STD (5) CONC-POS	[0] [0]
STD (6) CONC-POS	[0] [0]
UNIT	%
SD LIMIT	[999]
DUPLICATE LIMIT	[32000]
SENSITIVITY LIMIT	[0]
ABS LIMIT (INC/DEC)	[32000]-[INCREASE]
PROZONE LIMIT	[0] [LOWER]
EXPECTED VALUE	[*] [1]
PANIC VALUE	[-32000]-[32000]
INSTRUMENT FACTOR	[1.00]

\* **determined by user**  
\*\* **Conc. of calibrators**

### CALCULATION

HbA<sub>1c</sub> results are determined using calibration based on a suitable mathematical procedure and the Greiner Calibration Set.

For the 2-reagent procedure the calibration is stable for at least 1 day.

For the 3-reagent procedure the calibration is stable for at least 1 week.

*Check this by using Greiner's Control Set.*