HbA1c Protocol

Version: 1
Replaced by version
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Summary:

Direct Enzymatic HbA1c test is an enzymatic assay in which lysed whole blood samples are subjected to extensive protease digestion with Bacillus sp protease. This process releases amino acids including glycated valines from the hemoglobin beta chains. Glycated valines then serve as substrates for specific recombinant fructosyl valine oxidase (FVO) enzyme, produced in E. coli. The recombinant FVO specifically cleaves N-terminal valines and produces hydrogen peroxide. This, in turn, is measured using a horseradish per-oxidase (POD) catalyzed reaction and a suitable chromagen.

The HbA1c concentration is expressed directly as %HbA1c by use of a suit-able calibration curve in which the calibrators have values for each level in %HbA1c.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Calibrator	Diazyme	DZ168A-CAL
Reagents	Diazyme	DZ168A-K
Microplate		
Platereader		

Protocol:

1. Use 250 µl of lysis buffer to lyse 20 µl samples of whole blood and calibrators.

IMPORTANT: Make sure the samples are totally lysed. Any solid material floating around will interfere with reading in the platereader.

2. Mix R1a and R1b reagents together in a 70:30 ratio.

3. Add 25 µl of each calibrator and sample to each well.

4. Add 160 µl of reagent R1ab mix to each well. Incubate at 37°C for 5 minutes then read at 720 nm.

IMPORTANT: Make sure not to add any bubbles to the wells when dispensing reagents, this will interfere with reading in the platereader.

5. Add 70 µl of R2 to each well. Incubate at 37°C for 3 minutes then read at 720 nm.

6. Subtract blank readings from final readings. The assay will be linear so the unknown samples can be calculated as (Sample Absorbance ÷ Calibrator Absorbance) × Calibrator Concentration.

Reagent Preparation:

Lysis Buffer – ready to use
R1a & R1b – mix together in 70:30 ratio
R2 – ready to use