



UC Davis MMPC-Live Protocol

Hemoglobin A1C (HbA1C)

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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:

<i>Reagent/Material</i>	<i>Vendor</i>	<i>Stock Number</i>
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.
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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 μ l 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 $(\text{Sample Absorbance} \div \text{Calibrator Absorbance}) \times \text{Calibrator Concentration}$.

Reagent Preparation:

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