**Intended Use**

Direct Enzymatic Hemoglobin A1c (glycated hemoglobin A1c; HbA1c) reagents are intended for use in the quantitative determination of stable HbA1c in human whole blood samples. Measurement of hemoglobin A1c is a valuable indicator for long-term diabetic control. For in-vitro diagnostic use only.

**Clinical Significance**

Hemoglobin A1c is an important test recommended by the American Diabetes Association (ADA) and its usefulness was clarified by the United Kingdom Prospective Diabetes Study (UKPDS) and Diabetes Control and Complications Trial (DCCT). Currently, the HbA1c test is recommended for patients with diabetes every 2-3 months as part of the patient Diabetes management program.

**Assay Principle**

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 valine residues of hemoglobin beta chains. HbA1c test is used both as an index of mean glycemia and as a measure of risk for the development of diabetes complications. Therefore, the HbA1c test is a good indicator of glycemic control in the preceding 2-3 months.

**Reagent Composition**

**Lysis Buffer**

- CHES, pH 8.7: 100 mM
- Triton-X-100: 1%
- SDS: 0.45%
- Redox Agents: 0.5 mM

**Reagent R1a**

- MES pH 7.0: 5 mM
- Proteases: 4 KU/mL
- Triton-X-100: 0.5%
- Redox agents: >10µM

**Reagent R2a**

- MES pH 6.3: 1 mM
- Redox agent: <3 mM

**Reagent R2**

- Tris pH 8.0: 15 mM
- FVO enzyme: >10 IU/mL
- POD: 90 U/mL
- Chromagen: 0.8 mM

**Materials Required but not Provided**


**Reagent Preparation**

For analyzers capable of handling 3-reagents, Rta, Rtb, R2 are ready to use. For analyzers capable of handling only 2-reagents, N B S HbA1c reagents

Rta and Rtb should be mixed in a 2:3 ratio and allowed to sit at 2-8°C for 24 hours prior to use. To prepare sufficient Rtb mixture, pour the entire contents of Rtb bottle into Rta bottle. Mix gently by inversion.

**Reagent Stability and Storage**

Reagents are stable until their expiration date when stored at 2-8°C. Reconstituted Rtb thus prepared is stable for 4 weeks when stored at 2-8°C. Rtb and R2 reagents are light sensitive.

**Specimen Collection and Handling**

The assay is formulated for use with human whole blood samples. Venous whole blood samples collected with EDTA anticoagulant can be used. It is recommended that samples be used within 2 weeks of collection when stored refrigerated. Prior to testing, whole blood samples should be mixed by gentle inversion to re-suspend settled erythrocytes.

**Precautions**

Reagent Rtb and R2 are light-sensitive. Store in a dark place. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 938395). As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet. Do not use the reagents after the expiration date labeled on the outer box. Additional safety information concerning storage and handling of this product is provided in the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 949-309-2500.

**Whole Blood Bench Top Lysis Procedure**

Dispense 250 µL of Lysis reagent in a sample cup or an Eppendorf microfuge tube. Prior to testing, whole blood samples should be mixed by gently inversion at least 5 times to resuspend settled erythrocytes. Accuracy of the assay will be affected if whole blood is not thoroughly mixed prior to testing. Add 20 µL of fully suspended whole blood sample. Mix gently with a suitable pipettor without creating foam and incubate at room temperature (25°C) for 10 min to completely lyse the red blood cells. Complete lysis is observed when the mixture becomes a clear dark red solution without any particulate matter. Incubate the samples longer as needed to ensure complete hemolysate preparation. The lysate, thus prepared, is ready for use in the Direct Enzymatic HbA1c assay steps.

**Materials**


**Reagent Preparation**

For analyzers capable of handling 3-reagents, Rta, Rtb, R2 are ready to use. For analyzers capable of handling only 2-reagents, N B S HbA1c reagents

Rta and Rtb should be mixed in a 2:3 ratio and allowed to sit at 2-8°C for 24 hours prior to use. To prepare sufficient Rtb mixture, pour the entire contents of Rtb bottle into Rta bottle. Mix gently by inversion.

**Reagent Stability and Storage**

Reagents are stable until their expiration date when stored at 2-8°C. Reconstituted Rtb thus prepared is stable for 4 weeks when stored at 2-8°C. Rtb and R2 reagents are light sensitive.

**Specimen Collection and Handling**

The assay is formulated for use with human whole blood samples. Venous whole blood samples collected with EDTA anticoagulant can be used. It is recommended that samples be used within 2 weeks of collection when stored refrigerated. Prior to testing, whole blood samples should be mixed by gentle inversion to re-suspend settled erythrocytes.

**Precautions**

Reagent Rtb and R2 are light-sensitive. Store in a dark place. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 938395). As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet. Do not use the reagents after the expiration date labeled on the outer box. Additional safety information concerning storage and handling of this product is provided in the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 949-309-2500.

**Whole Blood Bench Top Lysis Procedure**

Dispense 250 µL of Lysis reagent in a sample cup or an Eppendorf microfuge tube. Prior to testing, whole blood samples should be mixed by gently inversion at least 5 times to resuspend settled erythrocytes. Accuracy of the assay will be affected if whole blood is not thoroughly mixed prior to testing. Add 20 µL of fully suspended whole blood sample. Mix gently with a suitable pipettor without creating foam and incubate at room temperature (25°C) for 10 min to completely lyse the red blood cells. Complete lysis is observed when the mixture becomes a clear dark red solution without any particulate matter. Incubate the samples longer as needed to ensure complete hemolysate preparation. The lysate, thus prepared, is ready for use in the Direct Enzymatic HbA1c assay steps.

**Materials**


**Reagent Preparation**

For analyzers capable of handling 3-reagents, Rta, Rtb, R2 are ready to use. For analyzers capable of handling only 2-reagents, N B S HbA1c reagents

Rta and Rtb should be mixed in a 2:3 ratio and allowed to sit at 2-8°C for 24 hours prior to use. To prepare sufficient Rtb mixture, pour the entire contents of Rtb bottle into Rta bottle. Mix gently by inversion.

**Reagent Stability and Storage**

Reagents are stable until their expiration date when stored at 2-8°C. Reconstituted Rtb thus prepared is stable for 4 weeks when stored at 2-8°C. Rtb and R2 reagents are light sensitive.

**Specimen Collection and Handling**

The assay is formulated for use with human whole blood samples. Venous whole blood samples collected with EDTA anticoagulant can be used. It is recommended that samples be used within 2 weeks of collection when stored refrigerated. Prior to testing, whole blood samples should be mixed by gentle inversion to re-suspend settled erythrocytes.

**Precautions**

Reagent Rtb and R2 are light-sensitive. Store in a dark place. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 938395). As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet. Do not use the reagents after the expiration date labeled on the outer box. Additional safety information concerning storage and handling of this product is provided in the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 949-309-2500.
Quality Control
RBI Direct Enzymatic HbA1c control set (DZ168A-CON) can be purchased separately. Users should follow the appropriate federal, state and local guidelines concerning the running of external quality controls and handling of bio-hazardous material. To ensure adequate quality control, level 1 and level 2 controls with known values should be run as unknown samples.

Results
The HbA1c concentration is expressed directly as %HbA1c by use of a suitable calibration curve in which the calibrators have values for each level in %HbA1c. The values reported are aligned with the Diabetes Control and Clinical Trials (DCCT) system and hence reported in the NGSP format. No calculation step is needed. The International Federation of Clinical Chemistry (IFCC) values can be calculated by use of published conversion formula: NGSP = [0.915 x (IFCC)] + 2.15.

Reference Range
Non-diabetic individuals have HbA1c values in the range of 3-6% and controlled diabetic individuals have HbA1c values in the 6-9% range. Individuals with uncontrolled diabetes can have HbA1c as high as 20%. The American Diabetes Association (ADA) recommends that the primary treatment goal in diabetes should be glucose control equal to that achieved during the DCCT. Based on DCCT, ADA states HbA1c targets of <7%. However, each laboratory must establish its own normal range in their country of business taking into account sex, age and ethnicity.

Limitations
The linearity of the assay is up to 16% HbA1c. Samples with values above 16% should not be diluted and retested. Instead the values should be reported as higher than 16% (>16%). The assay is formulated for use with human whole blood samples in EDTA. Total hemoglobin in the sample should be in the range: 9-21 g/dL. High HbF (>10%) may result in inaccurate HbA1c values.

Accuracy
The following HbA1c value data were obtained by comparing Diazyme Direct Enzymatic HbA1c assay to a legally marketed HPLC method.

<table>
<thead>
<tr>
<th>Whole blood application</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>44</td>
</tr>
<tr>
<td>Slope</td>
<td>1.0212</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0135</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9874</td>
</tr>
<tr>
<td>Range of values</td>
<td>5% - 13% HbA1c</td>
</tr>
</tbody>
</table>

Precision
Precision studies were conducted with the Diazyme Direct Enzymatic HbA1c assay reagents. Within-run and total precision studies were done by testing 2 levels of samples per NCCLS EP-5 procedure. Precision data is summarized in the table below:

<table>
<thead>
<tr>
<th>Level 1 (%HbA1c)</th>
<th>Level 2 (%HbA1c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value</td>
<td>5.7%</td>
</tr>
<tr>
<td>Within run SD (Swr)</td>
<td>0.06</td>
</tr>
<tr>
<td>Within run CV%</td>
<td>1.0%</td>
</tr>
<tr>
<td>Inter assay precision</td>
<td>0.10</td>
</tr>
<tr>
<td>Inter assay precision</td>
<td>1.8%</td>
</tr>
</tbody>
</table>

Linearity
RBI HbA1c assay has a linear range from 4.0% - 16.0%.

Interference
The assay is not affected by the following interfering substances at the indicated concentrations: ascorbic acid 12 mg/dL, total bilirubin 15 mg/dL, bilirubin (conjugated) 13 mg/dL, glucose 4000 mg/dL, triglyceride 4000 mg/dL, uric acid 30 mg/dL, urea 80 mg/dL. Stable glycosylated hemoglobin serves as a substrate for enzymatic reaction used in the RBI Direct Enzymatic HbA1c assay. Acetylated, carbamylated and labile HbA1c does not adversely affect the enzymatic reaction used in this assay. Variant hemoglobin S, C and E do not significantly interfere with RBI Direct Enzymatic HbA1c assay.

References
American Diabetes Association Clin. Practice recommendation, 1992, Diab Care 16S2 (93): 10-13
NGSP, http://www.missouri.edu/~diabetes/ngsp.html