**[Intended Use]**

*i-CROMA™ HbA1c* is an immunoassay system for quantitative measurement of Hemoglobin A1c in Human blood with *i-CROMA™ Reader*. The test is used for routine monitoring of the long-term glycemic status in patients with diabetes mellitus.

**[Summary and Test Principle]**

Glycated protein is formed post-translationally through the slow, nonenzymatic reaction between glucose and amino groups on proteins. HbA1c is a clinically useful index of mean glycemia during the preceding 120 days, the average life span of erythrocytes. Carefully controlled studies have documented a close relationship between the concentrations of HbA1c and mean glycemia. HbA1c is considered as a more reliable parameter in monitoring glycemia over the glycemic period of 120 days, the average life span of erythrocytes. Carefully controlled studies have documented a close relationship between the concentrations of HbA1c and mean glycemia.

*i-CROMA™ HbA1c* is based on the fluorescence immunoassay technology, specifically the competition immuno-detection method. Whole blood is added to the mixture of hemolysis buffer and detection buffer, which results in hemolysis of red blood cells. The mixture containing HbA1c from the hemolyzed red blood cells and fluorescence-labeled HbA1c peptides from detection buffer is loaded onto the sample well of the Cartridge. The mixture then migrates through the nitrocellulose matrix of the test strip by capillary action. HbA1c from the blood competes with fluorescence-labeled HbA1c peptides for binding sites on HbA1c antibodies fixed on the nitrocellulose matrix. As a result, the higher concentration of HbA1c produces a lower fluorescence signal from HbA1c peptides. The signal is interpreted and the result displayed on *i-CROMA™ Reader* in units of percentage.

**[Composition of reagents]**

*i-CROMA™ HbA1c* consists of Cartridge, Detection Buffer and Hemolysis Buffer.

- Cartridge contains mouse monoclonal anti-HbA1c antibodies and rabbit IgG immobilized on the test and the control lines of the strip, respectively.
- Detection Buffer contains fluorescence-labeled HbA1c-peptide, fluorescence-labeled anti-rabbit IgG, BSA as a stabilizer and sodium azide as preservative in PBS.
- Hemolysis Buffer is pre-dispensed individually in a small tube and composed of cationic detergent.

**[Materials provided]**

<table>
<thead>
<tr>
<th>REF</th>
<th>FPC-38</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Box contains:</strong></td>
<td></td>
</tr>
<tr>
<td>Cartridge</td>
<td>25 pouches</td>
</tr>
<tr>
<td>ID Chip</td>
<td>1 each</td>
</tr>
<tr>
<td>Insert</td>
<td>1 sheet</td>
</tr>
<tr>
<td>Detection Buffer*</td>
<td>1 vial</td>
</tr>
<tr>
<td>Hemolysis Buffer*</td>
<td>25 tubes</td>
</tr>
</tbody>
</table>

[* Packaged in Styrofoam box separately]

**[Materials required but not provided]**

- *i-CROMA™ Reader* *REF* FR-203
- *i-Chamber* [REF FPRR009]
- Thermal Printer (optional)
- Transfer pipette (75 and 100µL)
- Capillary tube (5 µL)
- *i-CROMA™ HbA1c* Control

**[Stability and Storage]**

- *i-CROMA™ HbA1c* Cartridge is stable for 20 months if stored at 4 - 30°C in its sealed pouch.
- Detection Buffer is stable up to 20 months if stored at 2 - 8°C.
- Hemolysis Buffer is stable up to 20 months if stored at 4 - 30°C in its sealed pouch.
- Do not freeze and avoid direct sunlight.

**[Sample collection and preparation]**

Capillary blood and venous blood with or without anticoagulants (EDTA, heparin and NaF) can be used. The whole blood specimen must be at room temperature and be homogeneous before testing. Fresh blood samples are recommended for best results, and samples over 24 hours after collection are to be avoided, if possible. If the specimens appear to be hemolyzed, another specimen should be obtained for testing.

**[Warning and precautions]**

- For In Vitro Diagnostic Use only.
- Do not use *i-CROMA™ HbA1c* after the expiry date.
- Do not interchange components from different lots.
- Detection buffer contains sodium azide (0.05%) which is a toxic agent.
- Allow Detection Buffer to reach room temperature (20-30°C) before starting a test.
- Use separate clean pipette tips for each specimen. Discard after single use.
- Blood specimens, used parts of this product, pipette tips and sample vials are potentially infectious. Proper laboratory safety techniques, handling and disposal methods should be followed in accordance with standard procedures and relevant regulations by microbiological hazard materials.
- *i-CROMA™ HbA1c* should not be used as the absolute evidence for diabetes mellitus. The results should be interpreted by a physician along with clinical findings and other laboratory test results.
- *i-CROMA™ HbA1c* is only operational in conjunction with *i-CROMA™ Reader* and *i-Chamber*. And tests should be applied by trained personnel working for the facility where the sample is taken.

**[Quality Control]**

- A quality control test using commercially available controls should be performed as a part of good testing practice, to confirm the expected QC results, to confirm the validity of the assay, and to assure the accuracy of patient results. If you want to perform QC of *i-CROMA™ HbA1c*, we recommend using Boditech Med Inc.’s *i-CROMA™ HbA1c* control.
- A quality control test should be performed at regular intervals, and before using a new kit with patient specimens. Controls should be tested to confirm the test procedure, and to verify the tests produce the expected QC results. QC specimens should also be run whenever there should arise any questions concerning the validity of results obtained. Upon confirmation of the expected results, the Cartridge is ready to use with patient specimens. Control standards are not provided with this *i-CROMA™ HbA1c*. For information about obtaining the controls, contact Boditech Med Inc.’s Technical Services for assistance.
[Limitation]

- The results of i-CHROMA™ HbA1c should be evaluated together with all clinical and laboratory data available. If HbA1c test result should not agree with the clinical evaluation, additional tests should be performed.
- Other factors may interfere with i-CHROMA™ HbA1c and may cause erroneous results. These include technical or procedural errors, as well as additional substances in blood specimens.

[Performance characteristics]

1. Interference testing/Specificity

Other bio-molecules, such as Gentisic acid (200mg/ml), Bilirubin (20mg/ml), Triglyceride (3000mg/dl), Ascorbic acid (5mg/dl) and Glucose (300mg/ml) were added to test specimen with much higher level than their physiological level in normal blood. There was no significant interference with the HbA1c measurement.

2. Comparability/Accuracy

The HbA1c concentrations of 150 clinical specimens were quantified independently with i-CHROMA™ HbA1c and Tosoh G7 (HPLC) methods. The test results displayed R² value of 0.9835 with respect to the value for Tosoh.

3. Precision

For the intra-assay imprecision, each control sample was tested with 10 replicates of the same lot on the same day for 3 different lots. And the inter-assays were performed on 5 sequential days, two runs per day, with 10 replicates at each control sample for 3 different lots.

<table>
<thead>
<tr>
<th>HbA1c (%)</th>
<th>Intra-assay</th>
<th>Inter-assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>5.4</td>
<td>5.43</td>
<td>0.12</td>
</tr>
<tr>
<td>9.7</td>
<td>9.85</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Note: Please refer to the table below to identify symbol

- Consult instructions for use
- Use by
- Batch code
- Catalog number
- Caution
- Do not reuse
- Manufacturer
- Sufficient for
- Authorised representative the European community
- In vitro diagnostic medical device
- Temperature limitation
- This product fulfills the requirements of Directive 98/79/EC on in vitro diagnostic medical device

For Technical Assistance Call
Boditech Med Inc.’s Technical Services at
Tel: +82-33-243-1400
E-mail: sales@boditech.co.kr

Rev. No.: 00
Rev. Date: 2012.11.07
# Procedure Guide

## i-CHROMA™ HbA1c Troubleshooting

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Message: “Hb Low”</strong></td>
<td>Hb concentration is less than 5.0g/dL, - Blood volume is less than 5 µL, - Blood is not fully mixed, - Air bubbles in the Hb window</td>
<td>- Use 5 µL blood sample and retest, - Shake the vial with blood sample 15 times vigorously and retest, - Follow the instruction to prevent bubble formation and retest, - If the error recurs, repeat the test with 10 µL blood sample</td>
</tr>
<tr>
<td><strong>Message: “Hb High”</strong></td>
<td>Hb concentration exceeds 25.0g/dL, - Blood volume exceeds 5 µL, - Foreign particles such as dust on the Hb window</td>
<td>- Use 5 µL blood sample and retest, - Keep the Hb window free of foreign particles and retest, - If the error recurs, the sample is not compatible with i-CHROMA™ HbA1c, A test with a HPLC based system is recommended</td>
</tr>
<tr>
<td><strong>High Result</strong></td>
<td>Air bubbles in Hb window</td>
<td>Follow the instruction to prevent bubble formation and retest.</td>
</tr>
<tr>
<td>Results are higher when compared with other instruments.</td>
<td>- i-CHROMA™ HbA1c calibrated against Tosoh G7, - Referencing instruments employing HPLC is recommended for comparison.</td>
<td></td>
</tr>
<tr>
<td>The result was read after the recommended 12 minutes</td>
<td>Read the result at 12 minutes after applying the mixture</td>
<td></td>
</tr>
<tr>
<td>The sample mixture was loaded after more than 1 minute after mixing</td>
<td>Load the mixture immediately after mixing</td>
<td></td>
</tr>
<tr>
<td>Test was run at the humidity level over 70%</td>
<td>Repeat the test at the humidity level below 70%</td>
<td></td>
</tr>
<tr>
<td><strong>Low Result</strong></td>
<td>Foreign particles are on the Hb window</td>
<td>Retest after cleaning the Hb window</td>
</tr>
<tr>
<td>Cartridge is exposed to the cold draft from the air conditioner.</td>
<td>Block the air circulation or relocate the instrument for a retest.</td>
<td></td>
</tr>
<tr>
<td>Results are lower when compared with other instruments</td>
<td>- i-CHROMA™ HbA1c calibrated against Tosoh G7, - Referencing instruments employing HPLC is recommended for comparison</td>
<td></td>
</tr>
<tr>
<td>Blood left in the capillary</td>
<td>Retest after shaking well enough to withdraw all the blood out the capillary</td>
<td></td>
</tr>
<tr>
<td>Result was read earlier than 12 minutes after applying the mixture.</td>
<td>Read the result at 12 minutes after applying the mixture</td>
<td></td>
</tr>
<tr>
<td><strong>Value error 3</strong></td>
<td>Tested without sample</td>
<td>Test with the sample</td>
</tr>
</tbody>
</table>

### Specification of i-CHROMA™ HbA1c

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Capillary whole blood</th>
<th>Venus blood with anticoagulant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Volume</td>
<td>5µL</td>
<td></td>
</tr>
<tr>
<td>Measuring Unit</td>
<td>% (HbA1c)</td>
<td>g/dL (Hemoglobin)</td>
</tr>
<tr>
<td>Incubation time</td>
<td>Pre-incubation time : 5 ~ 30 minutes</td>
<td>Incubation time : 12 minute</td>
</tr>
<tr>
<td>Measuring Range</td>
<td>Range : 4.0 ~ 15% (HbA1c) / 5.0 ~ 25.0 g/dL (Hemoglobin)</td>
<td>Measuring interval : 0.1% (HbA1c) / 0.1 g/dL (Hemoglobin)</td>
</tr>
<tr>
<td>Operation Range</td>
<td>Temperature : 20 ~ 30°C</td>
<td>Humidity : 10 ~ 70%</td>
</tr>
<tr>
<td>Reference Range</td>
<td>4.5 ~ 6.5%</td>
<td></td>
</tr>
</tbody>
</table>

### Precaution in use

1. Before starting the assay, the HbA1c cartridge needs conditioning in i-Chamber (5 ~ 30 minutes).
2. Watch for any air bubbles or foreign particles in window 1 after loading the sample mixture.
3. If stored in a refrigerator, allow 30 minutes or longer for cartridge to reach the room temperature, with the product pouch closed.
4. The mixture of Detection buffer and Hemolysis buffer must be used within 1 hour after mixing.
5. Do not open the Window 2 cover before the test.
6. If the Window 2 cover were opened, close it tight by sliding it away from the window 1.
7. Do not remove the device from the pouch until ready to use. The cartridge should be used immediately once opened.

### Description of i-CHROMA™ HbA1c Cartridge

1. **Window 1**: For Hb concentration - colorimetric method  
2. **Hb well**: 15µL mixture loading  
3. **A1c well**: 75µL mixture loading  
4. **Window 2**: For A1c concentration - fluorescence immunoassay technology  
   Window 2 is shielded with a sliding cover.
Value of i-CHROMA™ HbA1c can be adjusted via the local or the spot calibration. * Refer the local or the spot calibration manual.