**INTENDED USE**

The TRUEchemie HbA1c liquid reagent test kit is used for the direct quantitative determination of percent HbA1c (hemoglobin fraction) in human whole blood.

**INTRODUCTION**

Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin monomer increases in a predictable way. The test is limited to a three-month average because the lifespan of a red blood cell is four months (120 days). However, since RBCs do not all undergo lysis at the same time, HbA1c is taken as a limited measure of 3 months. In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, neuropathy, and retinopathy.

**PRINCIPLE**

The percentage of HbA1c in whole blood can be directly determined by utilizing the interaction of antigen and antibody. Total hemoglobin and HbA1c have the same unspecific absorption rate to latex particles. When we add mouse antihuman HbA1c monoclonal antibody (containing Multiple subunits), “latex--HbA1c--mouse antihuman HbA1c monoclonal antibody--HbA1c--latex” complex is formed. The amount of this complex is proportional to the amount of HbA1c absorbed on the surface of latex particles. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve.

**PACK SIZE**

<table>
<thead>
<tr>
<th>Kit Contents</th>
<th>5 x 1 ml</th>
<th>20 ml (1x20 ml)</th>
<th>40 ml (2x20 ml)</th>
<th>50 ml (1x50 ml)</th>
<th>50 ml (2x25 ml)</th>
<th>50 ml (3x15 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat No.</td>
<td>ADX941</td>
<td>ADX942</td>
<td>ADX943</td>
<td>ADX944</td>
<td>ADX945</td>
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</tr>
<tr>
<td>1) HbA1c Reagent-1 (R1)</td>
<td>1 x 15 ml</td>
<td>1 x 30 ml</td>
<td>1 x 30 ml</td>
<td>1 x 30 ml</td>
<td>1 x 30 ml</td>
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</tr>
<tr>
<td>2) HbA1c Reagent-2 (R2)</td>
<td>1 x 25 ml</td>
<td>1 x 50 ml</td>
<td>1 x 50 ml</td>
<td>1 x 50 ml</td>
<td>1 x 50 ml</td>
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</tr>
<tr>
<td>3) HbA1c Calibrators</td>
<td>5 x 1 ml</td>
<td>5 x 1 ml</td>
<td>5 x 1 ml</td>
<td>5 x 1 ml</td>
<td>5 x 1 ml</td>
<td></td>
</tr>
<tr>
<td>4) HbA1c Calibrators</td>
<td>5 x 1 ml</td>
<td>5 x 1 ml</td>
<td>5 x 1 ml</td>
<td>5 x 1 ml</td>
<td>5 x 1 ml</td>
<td></td>
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</tbody>
</table>

**REAGENTS COMPOSITION**

Reagent 1 (R1) Latex Reagent: Latex 0.13%, Buffer, Stabilizer Sodium azide (0.96 g/L)

Reagent 2 (R2) HbA1c Antibody: Buffer, Mouse anti-human HbA1c monoclonal antibody: 0.05 mg/ml, goat anti-mouse IgG polyvalent antibody 0.08 mg/ml, Stabilizers

Reagent 3 (R3) Hemolyzing reagent: Water and stabilizers.

**STORAGE AND STABILITY**

All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date. Store the Bottle tightly closed, protected from light and prevent contamination.

Do not centrifuge fresh/Non-frozen samples. Samples must be mixed thoroughly prior to use.

**SAMPLE STORAGE AND STABILITY**

Whole Blood samples and Hemolysed samples (lysate) are stable for 5 days at 2-8°C.

**SAMPLE PREPARATION**

1. Only below samples were tested and found suitable for use:
   - Dipotassium EDTA(K2-EDTA)
   - Lithium Heparin(Li-Heparin)
   - 0.13% Sodium azide
   - 0.05 mg/ml, goat anti-mouse IgG polyvalent antibody
2. Do not use samples that are heat-inactivated, pooled or with obvious microbial contamination.
3. Fresh/Non-Frozen samples: Do not centrifuge fresh/Non-frozen samples. Samples must be mixed thoroughly prior to use.
4. Frozen samples: a. Thaw the samples for a minimum of 30 minutes. b. Mix thawed samples thoroughly by inverting the vials until they are visibly homogeneous. c. To ensure consistency in results, frozen and thawed samples must be transferred to a centrifuge tube and centrifuge at >10,000 RCF (Relative Centrifugal Force) for 5 minutes before testing.
5. HEMOLYSE PREPARATION:
   - To determine the level of HbA1c, a hemolyse must be prepared for each sample:
     - a. Dispense 0.500 ml (500 µl) of Hemolyzing Solution (HbA1c Reagent -3 (R3)) into a tube.
     - b. Add 0.010 ml (10 µl) of well mixed whole blood sample.
     - c. Mix thoroughly by gentle vortexing for 30 seconds
     - d. Stand for 30 minutes at room temperature until complete lysis.
     - e. Take 0.010 ml (10 µl) of above lysate for testing.

**CALIBRATOR PREPARATION**

1. Reconstitute the calibrator with 1ml DI water.
2. Mix thoroughly by gentle vortexing for 30 seconds.
3. Stand for 30 minutes at room temperature and protect from light. Meanwhile, shake and flip over the brown bottle gently to ensure the content is fully dissolved. Avoid air bubbles.
4. Take 0.010 ml (10 µl) of reconstituted calibrator for testing.
5. Reconstituted calibrators are stable for 20 days at 2-8°C, and 30 days at -20°C.

**TEST PROCEDURE FOR PREPARATION OF CALIBRATION CURVE**

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>660 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>37°C</td>
</tr>
</tbody>
</table>

**Calculations:**

Δ O.D of Calibrator = O.D Calibrator - O.D Blank

**TEST PROCEDURE FOR PREPARATION OF SPECIMEN**

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>660 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>37°C</td>
</tr>
</tbody>
</table>

**Calculations:**

Δ O.D of Sample = O.D Sample - O.D Blank

**NORMAL VALUES**

- HbA1c Value: Glycaemic Goal
  - < 6% HbA1c: Less Strigent
  - 6% – 6.5% HbA1c: General (Non-Pregnant Adults)
  - > 6.5% HbA1c: More Stringent

It is strongly recommended that glycaemic goals are individualized following current professional society recommendations.
CALIBRATION

When using TRUEchemie HbA1c calibrators to do the multi-point calibration, the calibration cycle is 14 days. Re-calibration is required when changing reagent batch. It is recommended to use the normal & pathological quality control as internal quality control. Corrective action shall be done if controls do not recover within the acceptable tolerance.

LIMITATIONS

Linearity: up to 18.0 %
Sensitivity: 2%

INTERFERENCES

The assay is not affected by the following interfering substances at the indicated concentrations:
- Bilirubin up to 688 µ mol/L no interference effect
- Triglyceride up to 11.3 mmol/L no interference effect
- Ascorbic acid up to 0.5 g/L no interference effect

SYSTEMS PARAMETERS

<table>
<thead>
<tr>
<th>Mode</th>
<th>Endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
<td>--</td>
</tr>
<tr>
<td>Primary Wave length</td>
<td>660 nm</td>
</tr>
<tr>
<td>Linearity</td>
<td>2-18 %</td>
</tr>
<tr>
<td>Units</td>
<td>%</td>
</tr>
<tr>
<td>Flow cell Temp</td>
<td>37 °C</td>
</tr>
<tr>
<td>Blank</td>
<td>Reagent</td>
</tr>
<tr>
<td>Calibration type</td>
<td>Multi Standard Calibration</td>
</tr>
<tr>
<td>Reagent volume</td>
<td>0.300 ml (R1) + 0.100 ml (R2)</td>
</tr>
<tr>
<td>Sample volume</td>
<td>0.010 ml</td>
</tr>
<tr>
<td>Incubation</td>
<td>5 + 5 min.</td>
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<tr>
<td>Low Normal</td>
<td>3 %</td>
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<tr>
<td>High Normal</td>
<td>5.8 %</td>
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REFERENCES