4. Kit Contents

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>SORB</td>
<td>1</td>
<td>Microplate (ready to use) in foil packed coated with recombinant intrinsic factor. 12 x 8 Break-a-part wells.</td>
</tr>
<tr>
<td>CAL</td>
<td>6 x 1.5mL</td>
<td>Calibrators. Human sera ready to use anti-intrinsic factor level 0 AU/ml (1), 12.5 AU/ml (2), 25 AU/ml (3), 50 AU/ml (4), 100 AU/ml (5), 200 AU/ml (6). Calibrator 2 = Cut-off Calibrator (yellow cap). NHS/PBS/NaN3 (&lt;0.1% w/v).</td>
</tr>
<tr>
<td>CONTROL</td>
<td>1 x 1.5mL</td>
<td>Negative Control (red cap), ready to use (yellow solution, NHS/PBS/NaN3 (&lt;0.1% w/v)).</td>
</tr>
<tr>
<td>CONTROL+</td>
<td>1 x 1.5mL</td>
<td>Positive Control (green cap), ready to use (yellow solution, NHS/PBS/NaN3 (&lt;0.1% w/v)).</td>
</tr>
<tr>
<td>BUF+WASH</td>
<td>1 x 100mL</td>
<td>Wash Buffer Concentrate (20X) for 2L, (clear solution, PBST)</td>
</tr>
<tr>
<td>DIL+ SPE</td>
<td>1 x 50mL</td>
<td>Sample Diluent, ready to use (yellow solution, PBST/BSA/NaN3 (&lt;0.1% w/v)).</td>
</tr>
<tr>
<td>CONJ+ IFAB</td>
<td>1 x 15mL</td>
<td>Conjugate, ready to use (clear solution, anti-Hu IgG-HRP/MOPS/Proclin)</td>
</tr>
<tr>
<td>SUB+ TMB</td>
<td>1 x 15mL</td>
<td>TMB Substrate, black vial, ready to use (clear/bluish solution, TMB/H2O3)</td>
</tr>
<tr>
<td>STOP+</td>
<td>1 x 15mL</td>
<td>Stop Solution, white cap, ready to use (clear solution, 2.5% sulphuric acid)</td>
</tr>
</tbody>
</table>

5. Storage

The kit should be stored rehydrated at 2-8°C. Do not use the reagents beyond their expiry date. Do not freeze. Keep all reagents away from direct sunlight. Coated microwell strips are for one time use only. Unused microwell strips should be carefully resealed in the pouch containing desiccant to prevent condensation and stored at 2-8°C.

6. Sample Handling

AUTOZYME™ IFAB may be performed on human serum samples. Preferably, use freshly collected serum samples. Do not use citric, lipemic, haemolysed or bacterially contaminated samples. Sera with particles should be clarified by low speed centrifugation. Blood samples should be collected in additive-free tubes. After separation from the clot, the serum samples should be used immediately, respectively stored at 2-8°C for two or three days, or frozen at -20°C for longer periods.

7. Additional Reagents and Equipment Required

Deionised or freshly distilled water. Precision micropettes to deliver 5-1000µl. Multichannel micropipette or repeating dispenser to deliver 100µl. 2000ml measuring cylinders for reagent preparation. Microplate reader (450nm reading filter + optional 620nm reference filter). Automatic micropipe washer capable of dispensing 300µl. Automation - The AUTOZYME™ IFAB ELISA may be processed with suitable automated ELISA analysers. Applications have to be validated prior to diagnostic use.

8. Procedural Precautions

Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Allow all reagents to equilibrate to room temperature (18-25°C) before use. Do not mix or substitute reagents or microwells from different lot numbers. This may lead to variations in the results. Always change tips between different calibrators, samples or control sera to prevent sample carryover. Never allow the same pipette tip to be used with different reagents. Special care is needed to prevent contamination of the substrate by the conjugate. Protect the substrate material from light to avoid increase in blank values. The substrate solution should be colourless to a pale blue hue. Any blue colouration (OD >0.050 at 620nm) indicates substrate contamination and the substrate should be discarded.

The well washing procedure is critical for the successful performance of the test, especially between conjugate and substrate incubations (i.e. the second and third incubations) Do not use the kit beyond the expiry date given on the label. Unused reagents are stable at 2-8°C for up to 56 days after first opening the container. However, multiple re-use could increase the risk of reagent contamination.
9. **Assay Procedure**

1. Prepare the wash buffer as follows: dilute contents of the wash buffer concentrate (x20) to 2000ml with deionised water.

2. Dilute patient samples 1:100 using the sample diluent e.g. 5µl sample added to 495µl diluent. The kit calibrators and controls do not require dilution.

3. Remove the antigen-coated microwells from the resealable sachet. Reseal any unrequired wells in the resealable sachet, along with the desiccant sachet.

4. For semi-quantitative determination, pipette 100µl of each kit calibrator, control and diluted patient sample into the appropriate wells. For semi-quantitative determination, pipette 100µl kit calibrator 1 (blank), kit calibrator 2 (cut-off, yellow cap), controls and diluted patient samples in to the appropriate wells. Incubate for 30 minutes at room temperature (18°C to 25°C). It is recommended that calibrators and controls are tested in duplicate.

5. Discard the contents of the wells. Using the diluted wash buffer, wash the wells four (4) times with at least 300µl per well. Discard the contents of the wells and knock out the residue on absorbent material.

6. Pipette 100µl conjugate to each well and incubate for 30 minutes at room temperature.

7. Discard the contents of the wells. Wash the diluted wash buffer, wash the wells four (4) times with at least 300µl per well. Discard the contents of the wells and knock out the residue on absorbent material.

8. Pipette 100µl TMB substrate into each well and incubate for 10 minutes. At room temperatures above 25°C the substrate incubation could be shortened, but should never fall short of 5 minutes.

9. Stop the reaction by adding 100µl of stop solution.

10. Measure the OD at 450nm. Bi-chromatic measurement with a reference wavelength at 620nm is recommended. Read OD values within 30 minutes of adding Stop Solution.

11. **Quality Control**

Good laboratory practice requires that quality control specimens be included in every run to check on assay performance. The kit control ranges are provided on the certificate of analysis. If either control value falls outside the quoted range, the results are invalid and the assay should be repeated. Reagent blank (Calibrator 1) OD should be <0.05. Calibrator 6 OD > 1.6. Calibrator 2 (Cut-off) OD > Negative control OD < Positive control OD.

12. **Performance**

**Precision**

Samples measured twice per day for 20 days:

<table>
<thead>
<tr>
<th>Conc (AU/ml)</th>
<th>Std Dev</th>
<th>%CV</th>
<th>Between Day Std Dev</th>
<th>%CV</th>
<th>Between Run Std Dev</th>
<th>%CV</th>
<th>Within Device Std Dev</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.66</td>
<td>1.23</td>
<td>3.2</td>
<td>0.2</td>
<td>8.2</td>
<td>2.1</td>
<td>36.4</td>
<td>9.4</td>
<td>37.5</td>
</tr>
<tr>
<td>71.21</td>
<td>1.40</td>
<td>2.0</td>
<td>3.8</td>
<td>5.2</td>
<td>3.46</td>
<td>4.9</td>
<td>5.2</td>
<td>7.4</td>
</tr>
<tr>
<td>20.33</td>
<td>0.49</td>
<td>2.4</td>
<td>0.85</td>
<td>4.2</td>
<td>0.92</td>
<td>4.5</td>
<td>1.35</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Repeatability precision for 4 levels (n = 20):

Sample 1: Mean = 3.275AU/ml, Std Dev = 0.2525AU/ml, %CV = 7.7%
Sample 2: Mean = 40.573AU/ml, Std Dev = 0.8553AU/ml, %CV = 2.1%
Sample 3: Mean = 18.882AU/ml, Std Dev = 0.5629AU/ml, %CV = 3.0%
Sample 4: Mean = 74.567AU/ml, Std Dev = 3.0407AU/ml, %CV = 4.1%

**Clinical Sensitivity and Specificity**

The utility of the Intrinsic Factor Antibody ELISA was evaluated by testing 16 chronic anaemia patients alongside disease controls and ‘normal’ human sera. These results are summarised below:

- **Sensitivity** = 100.0%
- **Specificity** = 96.7%
- **Overall Agreement** = 99.0%
- **PPV** = 96.8%
- **NPV** = 100.0%

**LoD**

The limit of detection (LoD) was determined based on 60 replicates of the blank and 10 replicates of 6 low-level (NHS) samples. LoD was determined to be 0.1AU/ml.

13. **Safety Precautions**

For in vitro diagnostic use only and Professional Use only. Safety data sheets are available on request. This product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following recommendations and precautions for maximum safety when handling.

The kit contains potentially hazardous components. Reagents may be irritating to eyes and skin thus avoid contact with eyes and skin. Do not smoke, eat or drink when manipulating the kit.

The calibrators and controls contain human source material. Although found negative when tested for HIV-1 and HIV-2 antibodies, HCV and hepatitis B surface antigen, no test can guarantee their absence. Therefore, the calibrators and controls should be handled using the same safety precautions employed when handling any potentially infectious material. In case of contact with any reagent, immediately flush eyes or skin with water. If ingested, wash mouth with water and obtain medical attention immediately. Used calibrators, controls, samples, pipette tips and plates should be handled as clinical waste and incinerated or disposed of in accordance with local rules. Other reagents should be diluted and flushed down the drain. It is recommended that gloves are worn when handling such items.

**STOP**

Stop Solution contains 2.5% v/v Sulphuric acid.

Signal Word: Warning

Hazard Statements: H314 - Causes severe skin burns and eye damage.

Precautionary Statements: P280 - Wear protective goggles/ eye protection/face protection.
P305 + P351 + P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

14. **References**