Anti-Insulin-IgG EIA

For the quantitative and qualitative detection of antibodies against human insulin in human serum

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 35-INAHU-E01
Size: 96 wells
Version: 002 : 2007-08-28 - ALPCO 8/21/08
1. **Intended Use**

Anti-Insulin-G EIA is a solid phase enzyme immunoassay employing recombinant human insulin for the quantitative and qualitative detection of antibodies against human insulin in human serum. This kit is for research use only.

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**Principle of the test**

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the sample.
3. **Kit Contents**

*To be reconstituted:*

- **5x Sample Buffer**  
  1 vial, 20 ml - 5x concentrated (capped white: yellow solution)  
  Containing: Tris, NaCl, BSA, sodium azide <0.1% (preservative)

- **50x Wash Buffer**  
  1 vial, 20 ml - 50x concentrated (capped white: green solution)  
  Containing: Tris, NaCl, Tween 20, sodium azide <0.1% (preservative)

*Ready to use:*

- **Negative Control**  
  1 vial, 1.5 ml (capped green: colorless solution)  
  Containing: Human serum (diluted), sodium azide <0.1% (preservative)

- **Positive Control**  
  1 vial, 1.5 ml (capped red: yellow solution)  
  Containing: Human serum (diluted), sodium azide <0.1% (preservative)

- **Cut-off Calibrator**  
  1 vial, 1.5 ml (capped blue: yellow solution)  
  Containing: Human serum (diluted), sodium azide <0.1% (preservative)

- **Calibrators**  
  6 vials, 1.5 ml each 0, 3, 10, 30, 100, 300 U/ml  
  (color increasing with concentration: yellow solution)  
  Containing: Human serum (diluted), sodium azide <0.1% (preservative)

- **Conjugate**  
  1 vial, 15 ml IgG (capped blue: blue solution)  
  Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase

- **TMB Substrate**  
  1 vial, 15 ml (capped black)  
  Containing: Stabilized TMB/H2O2

- **Stop Solution**  
  1 vial, 15 ml (capped white: colorless solution)  
  Containing: 1M Hydrochloric Acid

- **Microplate**  
  12 x 8 well strips with breakaway microwells  
  Coating see paragraph 1

*Material required but not provided:*

Microplate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glassware (cylinder 100-1000 ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000 ml). Microplate washing device (300 µl repeating or multichannel pipette or automated system), absorbent paper. Our tests are designed to be used with purified water according to the definition of the US Pharmacopeia (USP 26-NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.)

4. **Storage and Shelf Life**

Store all reagents and the microplate at 2-8°C/35-46°F in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 4°C/39°F, at least. **Reagents and the microplate shall only be used within the expiry date indicated on each component. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.**
5. **Precautions of Use**

5.1 **Health hazard data**

This kit is for *Research Use Only. This kit is not for use in diagnostic procedures.* Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

*R e c o m m e n d a t i o n s a n d p r e c a u t i o n s*

This kit contains potentially hazardous components. Though kit reagents are not classified as being irritanting to eyes and skin, we recommend avoiding contact with eyes and skin and wearing disposable gloves. **WARNING:** Calibrators, Controls, and Buffers contain sodium azide (NaN₃) as a preservative. NaN₃ may be toxic if ingested or absorbed by skin or eyes. NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by the CDC or other local/national guidelines. Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

All human source material used for reagents of this kit (e.g. controls, standards) has been tested by approved methods and found to be negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and samples as if capable of transmitting infectious diseases and according to national requirements.

5.2 **General Directions for Use**

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

Never expose components to temperatures higher than 37°C/98°F.

Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

**Incubation:** **We recommend test performance at 30°C/86°F for automated systems.**

Always pipet substrate solution with only brand new tips. Protect this reagent from light. Never pipette conjugate with tips used previously with other reagents.

6. **Sample Collection, Handling and Storage**

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements.

Do not use icteric, lipemic, hemolyzed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (< 1000 x g). Blood samples should be collected in clean, dry, empty tubes. After separation, the serum samples should be used immediately, stored tightly closed at respectively 2-8°C/35-46°F for up to three days, or frozen at -20°C/-4°C for longer periods.
7. Assay Procedure

7.1 Preparations prior to pipetting
Dilute concentrated reagents:
Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).
Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Samples
Dilute serum samples 1:101 with sample buffer (1x).
e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well!

Washing
Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells.
e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:
Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:
Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean absorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice.

Microplates
Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

7.2 Work flow
For pipetting scheme see Annex A, for test procedure see Annex B. We recommend pipetting samples and calibrators in duplicate.

- Pipette 100 µl of each sample's diluted serum into the designated wells.
- Pipette 100 µl calibrators OR Cut off calibrator and negative and positive controls into the designated wells.
- Incubate for 30 minutes at room temperature (20-32°C/68-89.6°F).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl conjugate into each well.
- Incubate for 30 minutes at room temperature (20-32°C/68-89.6°F).
- Wash 3x with 300 µl washing buffer (diluted 1:50).
- Pipette 100 µl TMB substrate into each well.
- Incubate for 30 minutes at room temperature (20-32°C/68-89.6°F), protected from intense light.
- Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
- Incubate 5 minutes minimum.
- Agitate plate carefully for 5 sec.
- Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.
8. Quantitative and Qualitative Interpretation

For quantitative interpretation establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

<table>
<thead>
<tr>
<th>Normal Range</th>
<th>Equivocal Range</th>
<th>Positive Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 12 U/ml</td>
<td>12 - 18 U/ml</td>
<td>&gt; 18 U/ml</td>
</tr>
</tbody>
</table>

**Example of a standard curve**

We recommend pipetting calibrators in parallel for each run.

<table>
<thead>
<tr>
<th>Calibrators IgG</th>
<th>OD 450/620 nm</th>
<th>CV % (Variation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 U/ml</td>
<td>0.014</td>
<td>2.9</td>
</tr>
<tr>
<td>3 U/ml</td>
<td>0.138</td>
<td>1.0</td>
</tr>
<tr>
<td>10 U/ml</td>
<td>0.283</td>
<td>1.5</td>
</tr>
<tr>
<td>30 U/ml</td>
<td>0.620</td>
<td>2.3</td>
</tr>
<tr>
<td>100 U/ml</td>
<td>1.314</td>
<td>0.9</td>
</tr>
<tr>
<td>300 U/ml</td>
<td>2.074</td>
<td>1.2</td>
</tr>
</tbody>
</table>

**Example of calculation**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Replicate (OD)</th>
<th>Mean (OD)</th>
<th>Result (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 01</td>
<td>1.018/1.045</td>
<td>1.031</td>
<td>67.9</td>
</tr>
<tr>
<td>P 02</td>
<td>0.857/0.831</td>
<td>0.829</td>
<td>48.1</td>
</tr>
</tbody>
</table>

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and sample population according to its own established procedures.

For qualitative interpretation read the optical density of the cut-off calibrator and the samples. Compare sample OD with the OD of the cut-off calibrator. We recommend considering sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

- **Negative**: $\text{OD}_{\text{sample}} < 0.8 \times \text{OD}_{\text{cut-off}}$
- **Equivocal**: $0.8 \times \text{OD}_{\text{cut-off}} \leq \text{OD}_{\text{sample}} \leq 1.2 \times \text{OD}_{\text{cut-off}}$
- **Positive**: $\text{OD}_{\text{sample}} > 1.2 \times \text{OD}_{\text{cut-off}}$
9. Technical Data

Sample material: serum
Sample volume: 10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time: 90 minutes at (20-32°C/68-89.6°F)
Calibration range: 0-300 U/ml
Analytical sensitivity: 1.0 U/ml
Storage: at 2-8°C/35-46°F in original vials, only
Number of determinations: 96 tests

10. Performance Data

10.1 Analytical sensitivity
Testing sample buffer 30 times on the 35-7601 kit gave an analytical sensitivity of this kit has been found at 1.0 U/ml.

10.2 Specificity and sensitivity
The microplate is coated with recombinant human insulin. No crossreactivities to other autoantigens have been found. The diagnostic specificity of insulin antibodies is 98.5%. The diagnostic sensitivity of insulin antibodies is up to 70%. This data has been acquired with the 35-7601 kit.

Correlation:
The comparability of performance data was assessed with at least 30 sera tested on both 35-7601 and 35-3601. A linear regression analysis of the two products showed that the two products are equivalent. Data can be received upon request.

10.3 Linearity
Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Dilution Factor</th>
<th>measured concentration (U/ml)</th>
<th>expected concentration (U/ml)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 / 100</td>
<td>41.2</td>
<td>42.0</td>
<td>98.1</td>
</tr>
<tr>
<td></td>
<td>1 / 200</td>
<td>20.9</td>
<td>21.0</td>
<td>99.5</td>
</tr>
<tr>
<td></td>
<td>1 / 400</td>
<td>10.5</td>
<td>10.5</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>1 / 800</td>
<td>5.0</td>
<td>5.3</td>
<td>94.3</td>
</tr>
<tr>
<td>2</td>
<td>1 / 100</td>
<td>254.0</td>
<td>250.0</td>
<td>101.6</td>
</tr>
<tr>
<td></td>
<td>1 / 200</td>
<td>123.0</td>
<td>125.0</td>
<td>98.4</td>
</tr>
<tr>
<td></td>
<td>1 / 400</td>
<td>62.5</td>
<td>62.5</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>1 / 800</td>
<td>0.0</td>
<td>31.3</td>
<td>95.9</td>
</tr>
</tbody>
</table>
10.4 **Precision**
To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Mean (U/ml)</th>
<th>CV (%)</th>
<th>Sample No.</th>
<th>Mean (U/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45.3</td>
<td>4.6</td>
<td>1</td>
<td>37.7</td>
<td>3.9</td>
</tr>
<tr>
<td>2</td>
<td>8.7</td>
<td>9.4</td>
<td>2</td>
<td>8.8</td>
<td>8.4</td>
</tr>
<tr>
<td>3</td>
<td>235.6</td>
<td>2.9</td>
<td>3</td>
<td>314.8</td>
<td>5.1</td>
</tr>
</tbody>
</table>

10.5 **Calibration**
Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

11. **Literature**
ANNEX A: Pipetting scheme

We suggest pipetting calibrators, controls and samples as follows:

For **quantitative interpretation** use calibrators to establish a standard curve.

For **qualitative interpretation** use cut-off calibrator.

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
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<tbody>
<tr>
<td>A</td>
<td>CalA</td>
<td>CalE</td>
<td>P1</td>
<td></td>
<td>NC</td>
<td>P2</td>
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</tr>
<tr>
<td>B</td>
<td>CalA</td>
<td>CalE</td>
<td>P1</td>
<td></td>
<td>NC</td>
<td>P2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>CalB</td>
<td>CalF</td>
<td>P2</td>
<td></td>
<td>CC</td>
<td>P3</td>
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<tr>
<td>D</td>
<td>CalB</td>
<td>CalF</td>
<td>P2</td>
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<td>CC</td>
<td>P3</td>
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<tr>
<td>E</td>
<td>CalC</td>
<td>PC</td>
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<td>F</td>
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<td>PC</td>
<td>P3</td>
<td></td>
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<td>G</td>
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<td>NC</td>
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</tbody>
</table>

**CalA**: calibrator A, **CalB**: calibrator B, **CalC**: calibrator C, **CalD**: calibrator D, **CalE**: calibrator E, **CalF**: calibrator F
**PC**: positive control
**NC**: negative control
**CC**: Cut-off calibrator
**P1**: Sample 1
**P2**: Sample 2
**P3**: Sample 3