Anti-tTg-A (Anti Tissue Transglutaminase) ELISA

For the quantitative and qualitative detection of IgA antibodies against tTG in human serum.

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

Catalog Number: 35-TGAHU-E01
Size: 96 wells
1. **Intended Use**

The **tTG-A ELISA** of the new generation is a solid phase enzyme immunoassay for the quantitative and qualitative detection of antibodies against neo-epitopes of tissue transglutaminase (tTG) in human serum. The assay employing human recombinant transglutaminase crosslinked with gliadin-specific peptides displays neo-epitopes of tTg which ensures a significant increased sensitivity and specificity of the test.

2. **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), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)

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

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

- **Conjugate, IgA**
  - 1 vial, 15 ml IgA (capped red: red solution)
  - Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)

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

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

- **Microtiterplate**
  - 12x8 well strips with breakaway microwells
  - Coating. See paragraph 1.
**Material required but not provided:**
- Microtiter plate reader 4510 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 µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States 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 at 2-8°C/35-46°F for at least 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. 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 PRODUCT IS FOR RESEARCH USE ONLY! Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

*Recommendations and precautions*
This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin it is recommended to avoid contact with eyes and skin and wear disposable gloves.

**WARNING !** Calibrators, Controls and Buffers contain sodium azide (NaN₃) as a preservative. NaN₃ may be toxic if ingested or adsorbed 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 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 some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found 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.

The kit contains material of animal origin as stated in the table of contents, handle 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.

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.**

Never expose components to higher temperature than 37°C/ 98.6 °F.

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

6. **Sample Collection, Handling and Storage**
Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements.
Do not use icteric, lipemic, hemolysed 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 and empty tubes. After separation, the serum samples should be used during the first 8 hours, respectively stored tightly closed at 2-8°C/35-46°F up to 48 hours, or frozen at -20°C/-4°F 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 adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

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 the test procedure see Annex B

It is recommended to pipette samples and calibrators in duplicate.

Cut-off calibrator should be used for qualitative testing only.

- Pipette 100 µl of each diluted serum into the designated microwells.
- Pipette 100 µl calibrators (quantitative) OR cut-off calibrator (qualitative) and negative and positive controls into the designated wells.
- Incubate for 30 minutes at 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 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 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 it is recommended log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in **U/ml**.

**Example of a standard curve**

It is recommended to pipette calibrators in parallel for each run.

<table>
<thead>
<tr>
<th>Calibrators IgA</th>
<th>OD 450/620 nm</th>
<th>CV % (Variation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 U/ml</td>
<td>0.073</td>
<td>3.1</td>
</tr>
<tr>
<td>3 U/ml</td>
<td>0.179</td>
<td>2.3</td>
</tr>
<tr>
<td>10 U/ml</td>
<td>0.342</td>
<td>1.2</td>
</tr>
<tr>
<td>30 U/ml</td>
<td>0.662</td>
<td>0.1</td>
</tr>
<tr>
<td>100 U/ml</td>
<td>1.310</td>
<td>0.9</td>
</tr>
<tr>
<td>300 U/ml</td>
<td>2.263</td>
<td>0.3</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>01</td>
<td>0.808/0.831</td>
<td>0.820</td>
<td>39.6</td>
</tr>
<tr>
<td>02</td>
<td>1.081/1.071</td>
<td>1.076</td>
<td>66.1</td>
</tr>
</tbody>
</table>

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

Each laboratory should establish its own range based upon its own techniques, controls, equipment according to their own established procedures.

For qualitative interpretation read the optical density of the cut-off calibrator and the samples. Compare OD with the sample OD of the cut-off calibrator.

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 use original vials, only
**Number of determinations:** 96 tests

10. Performance Data

**10.1 Analytical sensitivity**

Testing sample buffer 30 times on this *tTg-A* ELISA gave an analytical sensitivity of 1.0 U/ml.
10.2 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>76.5</td>
<td>71.0</td>
<td>107.7</td>
</tr>
<tr>
<td></td>
<td>1 / 200</td>
<td>36.6</td>
<td>35.5</td>
<td>103.1</td>
</tr>
<tr>
<td></td>
<td>1 / 400</td>
<td>17.2</td>
<td>17.8</td>
<td>96.8</td>
</tr>
<tr>
<td></td>
<td>1 / 800</td>
<td>8.5</td>
<td>8.9</td>
<td>95.8</td>
</tr>
<tr>
<td>2</td>
<td>1 / 100</td>
<td>62.2</td>
<td>59.0</td>
<td>105.4</td>
</tr>
<tr>
<td></td>
<td>1 / 200</td>
<td>29.7</td>
<td>29.5</td>
<td>100.7</td>
</tr>
<tr>
<td></td>
<td>1 / 400</td>
<td>13.3</td>
<td>14.8</td>
<td>90.2</td>
</tr>
<tr>
<td></td>
<td>1 / 800</td>
<td>7.0</td>
<td>7.4</td>
<td>94.9</td>
</tr>
</tbody>
</table>

10.3 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>Intra-Assay</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Inter-Assay</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample No.</td>
<td>Mean (U/ml)</td>
<td>CV (%)</td>
<td>Sample No.</td>
<td>Mean (U/ml)</td>
<td>CV (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13.8</td>
<td>7.0</td>
<td>1</td>
<td>10.1</td>
<td>2.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>56.7</td>
<td>5.2</td>
<td>2</td>
<td>38.9</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>166.5</td>
<td>5.5</td>
<td>3</td>
<td>169.4</td>
<td>4.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10.4 Calibration

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

11. Literature

   Identification of tissue transglutaminase as the autoantigen of celiac disease.
   Nat Med 3: 797-801.

   Autoantibodies to tissue transglutaminase as predictors of celiac disease.
   Gastroenterology 115: 1317-1321.

   Lancet 349: 1755-1759.

5. Logan RFA. (1992) Problems and pitfalls in epidemiological studies of celiac disease


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>for quantitative interpretation use calibrators to establish a standard curve</th>
<th>for qualitative interpretation use cutoff calibrator</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CalA                              CalE                              S1</td>
<td>NC       S2</td>
</tr>
<tr>
<td>B</td>
<td>CalA                              CalE                              S1</td>
<td>NC       S2</td>
</tr>
<tr>
<td>C</td>
<td>CalB                              CalF                              S2</td>
<td>CC       S3</td>
</tr>
<tr>
<td>D</td>
<td>CalB                              CalF                              S2</td>
<td>CC       S3</td>
</tr>
<tr>
<td>E</td>
<td>CalC                              PC                                 S3</td>
<td>PC       ...</td>
</tr>
<tr>
<td>F</td>
<td>CalC                              PC                                 S3</td>
<td>PC       ...</td>
</tr>
<tr>
<td>G</td>
<td>CalD                              NC                                 ...</td>
<td>S1       ...</td>
</tr>
<tr>
<td>H</td>
<td>CalD                              NC                                 ...</td>
<td>S1       ...</td>
</tr>
</tbody>
</table>

PC: positive control
NC: negative control
CC: Cut-off calibrator
S1: Sample 1
S2: Sample 2
S3: Sample 3
Annex B: Test Procedure

1. Samples (1:101) / Controls

   - +100 µl
   - 30’
   - 3x 300µl

2. CONJ

   - +100 µl
   - 30’
   - 3x 300µl

3. SUB

   - +100 µl
   - 30’
   - +100 µl
   - 5’
   - 450 nm

STOP

\[ \text{OD}_{450} \]