Arginase Liver Type Human ELISA

Cat. No.: DEIA4501
Pkg. Size: 96T

**Intended use**

The Human Arginase Liver-Type ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Arginase I (Liver-Type).

**General Description**

Arginase [EC 3.5.3.1; L-arginine aminohydrolase] is an enzyme that hydrolyzes L-arginine to L-ornithine and urea in the urea cycle. Two forms of arginase exist which are designed as arginase I and arginase II. Liver-type arginase is expressed primarily in the liver and to some extent in the erythrocytes. Arginase II is expressed in many extrahepatic tissues, such as brain, spinal cord, kidney, small intestine and mammary gland. Although arginase I and arginase II have similar enzyme activities, they have different pI, immunological reactivity and are encoded by different genes. Human arginase I is a 35 kDa protein circulating in blood probably as a homotrimer. Circulating liver-type arginase was clinically used as a liver specific marker which may reflect not only early occurrence of liver injury but also early termination of liver injury. The measurement of liver-type arginase is clinically applicable for monitoring conditions of patients with liver disorders or postoperative conditions of patients who received partial hepatectomy with quicker normalization in comparison with aminotransferases (ALT and AST). Recently, arginase I gene was found to be one of the most prominent among asthma genes. In situ hybridization demonstrated marked staining of arginase I in submucosal inflammatory lesions and arginase activity is increased in allergen challenged lungs. Finally, it was found that both arginase I was the most significantly up-regulated protein in the murine spinal cord during experimental autoimmune encephalomyelitis. The results indicated that arginase played important roles in autoimmune inflammation in the central nervous system.

**Principle Of The Test**

In the Human Arginase Liver Type ELISA Standards, Quality Controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human arginase antibody. After 60 minutes incubation and washing, monoclonal anti-human arginase antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured arginase. Following another washing step, the remaining HRP conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of arginase. A standard curve is constructed by plotting absorbance values against concentrations of Standards and concentrations of unknown samples are determined using this standard curve.

**Reagents And Materials Provided**

- Antibody Coated Microtiter Strips: 96 wells
- Conjugate Solution: 13 ml
- Master Standard: 2 vials
- Quality Control HIGH: 2 vials
- Quality Control LOW: 2 vials
- Standard Diluent: 2 ml
- Dilution Buffer: 13 ml
- Wash Solution Conc. (10x): 100 ml
Substrate Solution: 13 ml
Stop Solution: 13 ml
Product Data Sheet + Certificate of Analysis: 1 pc

**Materials Required But Not Supplied**

1. Deionized (distilled) water
2. Test tubes for diluting samples
3. Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
4. Precision pipettes to deliver 5-1000 μl with disposable tips
5. Multichannel pipette to deliver 100 μl with disposable tips
6. Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
7. Vortex mixer
8. Orbital microplate shaker capable of approximately 300 rpm
10. Microplate reader with 450±10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
11. Software package facilitating data generation and analysis (optional)

**Storage**

2–8 °C/Wet ice

**Specimen Collection And Handling**

The kit measures human Arginase I (Liver-Type) in serum and cerebrospinal fluid (CSF).

**Stability and storage:**

Samples should be assayed immediately after collection or should be stored at -20°C, or preferably at -70°C (then the stability is at least 1 year). Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples. Do not store the diluted samples.

**Sera:**

It is recommended to spin the erythrocytes down immediately (within few seconds) after taking blood. It is not possible to get reliable results when measuring arginase in normal serum. Trace hemolysis and contamination of serum with erythrocyte arginase causes false increased results

Dilute serum samples just prior to the assay 4x with Dilution Buffer, e.g. 60 μl of sample + 180 μl of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

**CSF:**

Dilute CSF samples just prior to the assay 2x with Dilution Buffer, e.g. 120 μl of sample + 120 μl of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

**Reagent Preparation**

All reagents need to be brought to room temperature prior to use. Always prepare only the appropriate quantity of reagents for your test. Do not use components after the expiration date marked on their label.

**Assay reagents supplied ready to use:**

Antibody Coated Microliter Strips

**Stability and storage:**

Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips
are stable 3 months stored at 2-8°C and protected from the moisture.

Conjugate Solution
Standard Diluent
Dilution Buffer
Substrate Solution
Stop Solution

Stability and storage:
Opened reagents are stable 3 months when stored at 2-8°C.

**Assay reagents supplied concentrated or lyophilized:**

Human Arginase Master Standard
Reconstitute the lyophilized Master Standard with 150 μl of deionized (distilled) water just prior to the assay. Let it dissolve for 15 minutes and mix thoroughly. The resulting concentration of the human arginase in the stock solution is 320 ng/ml.

Prepare set of standards using Standard Diluent as follows:
Dilute prepared Standards 4x with Dilution Buffer prior to the assay, e.g. 60 μl of Standard + 180 μl of Dilution Buffer for duplicates.

Stability and storage:
Stability of the reconstituted Master Standard and diluted Standards is limited; they have to be prepared just before the use in ELISA (within 30 min).

Do not store the diluted Standard solutions.

**Quality Controls HIGH, LOW**
Reconstitute each Quality Control (HIGH and LOW) with 60 μl of deionized (distilled) water just prior to the assay. Let it dissolve for 15 minutes and mix thoroughly.
Dilute Quality Controls 4x with Dilution Buffer prior to the assay, e.g. 60 μl of Control + 180 μl of Dilution Buffer for duplicates.

Stability and storage:
The reconstituted Quality Controls must be used immediately or aliquoted and frozen at -20°C for 3 month. Avoid repeated freeze/thaw cycles.

Do not store the diluted Quality Controls.

**Wash Solution Conc. (10x)**
Dilute Wash Solution Conc. (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Conc. (10x)+ 900 ml of distilled water for use of all 96-wells. Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Conc. (10x) is stable 3 months when stored at 2-8°C.

**Assay Steps**

1. Pipet 100 μl of diluted Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells.
2. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add 100 μl of Conjugate Solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add 100 μl of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for 10 minutes at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction...
temperature is below than 20°C. Do not shake the plate during the incubation.
9. Stop the colour development by adding 100 μl of Stop Solution.
10. Determine the absorbance by reading the plate at 450 nm. (Optionally, to measure in dual wavelength mode 620-650 nm filter can be used to measure the reference absorbance. The absorbance should be read within 5 minutes following step 9).
Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine arginase concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

Calculation

1. Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance at 450 nm (Y) of Standards against log of the known concentration (X) of Standards, using the four-parameter algorithm. Results are reported as concentration of human Arginase I (Liver-Type) ng/ml in samples.
2. Alternatively, the logit log function can be used to linearize the standard curve (i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.
3. Serum samples, Quality Controls and Standards are all diluted 4x prior to the analysis, so there is no need to take this dilution factor into account.
4. The measured concentration of cerebrospinal fluid samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted 2x prior to the assay, while standards and Quality Controls are diluted 4x (e.g. 8.75 ng/ml (from standard curve) x 0.5 (dilution factor) = 4.375 ng/ml).

Typical Standard Curve

Typical Standard Curve for Human Arginase Liver Type ELISA
**Detection Range**

It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for human Arginase I (Liver-Type) levels with the assay.

**Sensitivity**

Analytical Limit of Detection is calculated from the real human arginase liver-type values in wells and is 0.5ng/ml

**Specificity**

Since Arginase I (Liver-Type) exists also in erythrocytes, the erythrocyte-derived arginase cross-reacts and hemolytic sera cannot be used in this assay. Low concentrations of the erythrocyte-derived arginase in apparently non-hemolytic sera can be subtracted. The erythrocytes have to be spun down immediately (within few seconds) after taking blood to avoid hemolysis and contamination of the sample with erythrocyte Arginase.

The antibodies used in this ELISA are specific for natural and recombinant human Arginase I (Liver-Type). No cross-reactivity has been observed for human Arginase II.
### Linearity

Serum samples (4x diluted) were serially diluted with Dilution Buffer and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (ng/ml)</th>
<th>Expected (ng/ml)</th>
<th>Recovery O/E (%)</th>
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### Recovery

Serum samples were spiked with different amounts of human Arginase I (Liver-Type) and assayed.

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<th>Observed (ng/ml)</th>
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<th>Recovery O/E (%)</th>
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### Reproducibility

Intra-assay (Within-Run) (n=8), CV: 4.9%-6.7%

Inter assay (Run-to-Run) (n=8), CV: 7.3%-8.6%

### Precautions

1. Wear gloves and laboratory coats when handling immunodiagnostic materials.
2. Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
3. This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV
1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents.

4. Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary.

5. The materials must not be pipetted by mouth.

**Analyte Gene Information**

<table>
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<tr>
<th>Gene Name</th>
<th>ARG1 arginase, liver [ Homo sapiens ]</th>
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**Pathway**

ATF-2 transcription factor network, organism-specific biosystem; Amoebiasis, organism-specific biosystem; Amoebiasis, conserved biosystem; Arginine and proline metabolism, organism-specific biosystem; Arginine and proline metabolism, conserved biosystem; IL4-mediated signaling events, organism-specific biosystem; Metabolic pathways, organism-specific biosystem;

**Function**

arginase activity; hydrolase activity; manganese ion binding; metal ion binding;

**REFERENCES**
