

Human Arginase I (Liver-Type) ELISA

Cat. No. RD193028000R

Manufacturer

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Use only the actual version of Product Data Sheet enclosed with the kit!

1. Intended Use

The RD193028000R Human Arginase Liver-Type ELISA is a double monoclonal sandwich enzyme immunoassay for the quantitative measurement of human liver-type arginase in serum and cerebrospinal fluid (CSF). It is intended for research use.

Features

- The total assay time is less than three hours.
- The kit measures total serum liver-type arginase.
- Quality controls are human serum based. No animal sera are used.
- Serum samples require very careful preparation. The erythrocytes have to be spinned down immediately (within few seconds) after taking blood to avoid hemolysis and contamination of the sample with erythrocyte arginase.

2. Storage, Expiration

Place the lyophilized Master Standards and Quality Controls at –20 °C after the kit delivery.

Store the other kit components at 2-8°C.

Under these conditions the kit is stable till the expiry date is over. (See the expiry date indicated on the kit label).

3. Summary

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 designated as arginase I and arginase II. Liver-type arginase I 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 pre- and 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 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 I played important roles in autoimmune inflammation in the central nervous system.

4. Test Principle

In the BioVendor's Human Arginase Liver-Type ELISA, calibrators or samples are incubated with a monoclonal anti-human arginase antibody coated in microtiter wells. The wells are washed and horseradish-labelled monoclonal anti-human arginase antibody (conjugate) is added and incubated with captured arginase. After a thorough wash, the conjugate bound is allowed to react with the substrate (H₂O₂-tetramethylbenzidine). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of arginase. A standard curve is constructed by plotting

absorbance values versus arginase concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

5. Precautions

- For research use only.
- This kit contains components of human origin. These materials were found non-reactive for HbsAg, HCV antibody and for HIV 1/2 antibody and antigen. However, these materials should be handled as potentially infectious, as no tests can guarantee the complete absence of infectious agents.
- Wear gloves and laboratory coats when handling immunodiagnostic materials and samples.
- Avoid contact with the acid Stop Solution and Substrate Solution, which contains hydrogen peroxide. Wear gloves and eye protection when handling these reagents. In case of contact with the Stop Solution and the Substrate Solution wash skin thoroughly with water and seek medical attention, when necessary.
- The materials must not be pipetted by mouth.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- Reagents with different lot numbers should not be mixed.
- Reagents should not be used after the expiry date specified on the kit label.

6. Reagents Supplied

<i>Cat. No.</i>	<i>Kit Components</i>	<i>Quantity</i>
C121211	Microtiter Strips, coated with capture Anti-Arginase Antibody, sealed	96 wells
C122211	Conjugate Solution (Anti-Arginase Antibody, Horseradish Peroxidase Conjugate), ready to use	13 ml
C123142	Human Liver-Type Arginase Master Calibrator (320 ng/ml); lyophilized	2 vials
C124151	Quality Control High, lyophilized	2 vials
C124251	Quality Control Low, lyophilized	2 vials
C125711	Calibrator Diluent	2 ml
C005111	Dilution Buffer, ready to use	13 ml
C006121	Wash Solution Concentrate (10x)	100 ml
C007111	Substrate Solution (TMB), ready to use	13 ml
C008111	Stop Solution (0.2 M H ₂ SO ₄) ready to use	13 ml

7. Materials Required but Not Supplied

- Test tubes for diluting samples
- Precision pipettes to deliver 10-1000 μ l and disposable tips
- Multichannel pipette 100 μ l and disposable tips
- Microplate reader with 450 ± 10 nm filter
- Software package facilitating data generation and analysis
- Orbital microplate shaker capable of agitation at approximately 300 rpm (optional)
- Microtitration plate washer (optional). [Manual washing is possible but not preferable.]
- Absorbent material for blotting the microtiter plate
- Glassware (graduated cylinder and bottle for Wash Solution)
- Deionized (distilled) water

8. Preparation of Reagents

All reagents need to be brought to room temperature prior to the assay.

Assay reagents are supplied ready-to-use, with the exception of Decoy Receptor 3 Master Calibrator, Quality Controls and Wash Solution Concentrate (10x).

- If you do not use the whole plate, return unused strips in the provided aluminium bag with dessicant and seal the bag carefully. Keep the unused strips at 2-8°C, protected from the moisture.

Preparation of reagents for 1 plate:

Wash Solution:

Dilute 100 ml of Wash Solution Concentrate with 900 ml of deionized (distilled) water. The working Wash Solution is stable for 1 month at 2-8 °C.

Reconstitution of Master Calibrator, Preparation, and Dilution of Standard Solutions:

The preparation of standards consists of three steps.

Reconstitute the lyophilized Master Calibrator: Add 150 µl of deionized (distilled) water to the vial containing lyophilized Master Standard, let it dissolve for 10-15 minutes and mix thoroughly.

Prepare all concentrations of arginase standards: Dilute the reconstituted Master Standard with Standard Diluent as described below:

<i>Volume of standard</i>	<i>Standard Diluent</i>	<i>Concentration</i>
Master	-----	320 ng/ml
75 µl of Master	75 µl	160 ng/ml
75 µl of Std. 160 ng/ml	75 µl	80 ng/ml
75 µl of Std. 80 ng/ml	75 µl	40 ng/ml
75 µl of Std. 40 ng/ml	75 µl	20 ng/ml
75 µl of Std. 20 ng/ml	75 µl	10 ng/ml
75 µl of Std. 10 ng/ml	75 µl	5 ng/ml

Dilute the standards: Dilute the freshly prepared Standards 1:4 with Dilution Buffer prior to use (preferably 60 µl Standard + 180 µl Dilution Buffer).

Reconstitution of Quality Controls: Add 60 µl of deionized (distilled) water to the vial containing a lyophilized Quality Control, let it dissolve for at least 15 minutes and mix thoroughly. The reconstituted control serum has to be used immediately or to be stored frozen.

Dilution of Quality Controls: Dilute the reconstituted Quality Control 1:4 with Dilution Buffer (preferably 60 µl Control + 180 µl Dilution Buffer).

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).

9. Preparation of 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

Storage: Samples should be stored frozen (preferably at -80 °C, then the stability is at least 1 year).

Repeated thawing-freezing cycles should be avoided.

Dilution of Sera: Dilute serum samples 1:4 with Dilution Buffer (preferably 60 μ l sample + 180 μ l Dilution Buffer for duplicates).

Dilution of CSF: Dilute CSF samples 1:2 with Dilution Buffer (preferably 120 μ l sample + 120 μ l Dilution Buffer for duplicates).

10. Assay Procedure

- 1) Pipet 100 μ l of diluted Standards, Quality Controls and samples, preferably in duplicates, into the appropriate wells.
- 2) Incubate the plate for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker at RT.
- 3) Wash the wells 3-times with Wash Solution (0.35 ml per well).
- 4) Add 100 μ l of Antibody-HRP Conjugate solution.
- 5) Incubate the plate for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker at RT.
- 6) Wash the wells 3-times with Wash Solution (0.35 ml per well).
- 7) Add 100 μ l of Substrate Solution. (Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.)
- 8) Incubate the plate for 10 min without shaking at RT.
- 9) Stop the colour development by adding 100 μ l 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.

11. Calculations

Most microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the absorbance (Y) of standards versus *log* of the known concentration (X) of standards, using the four-parameter function. Results are reported as concentration of arginase (ng/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the calibration curve (i.e. *logit* of absorbance (Y) is plotted versus *log* of the known concentration (X) of standards). Use the dilution factor of 0.5 to obtain real concentrations in cerebrospinal fluid samples (diluted 1:2).

12. Limits of Assay

Results exceeding 320 ng/ml should be repeated with more diluted samples (e.g. 1:8). Dilution factors (e.g. 2) need to be taken into consideration in calculating the Arginase concentration.

13. Performance Characteristics

Typical analytical data of BioVendor Human Arginase Liver-Type ELISA are presented in this chapter.

For actual Standard curve and Quality Controls values see the Certificate of Analysis.

- **Sensitivity**

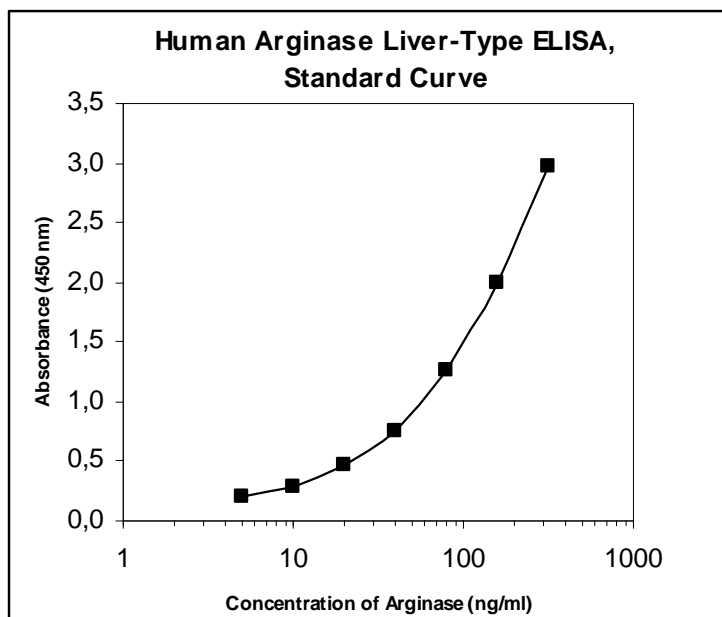
The limit of detection (defined as human arginase liver-type concentration giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$) is defined as follows:

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

Assay Sensitivity takes the dilution of samples into consideration and is calculated according to the formula:

Assay Sensitivity = Analytical Limit of Detection x sample dilution = 0.17ng/ml x 4 = 2.0ng/ml

*Dilution Buffer is pipetted into blank wells.



- **Specificity**

Since Arginase I exist also in erythrocytes, the erythrocyte-derived arginase cross-reacts and haemolytic 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 assay determines natural and recombinant human Liver-Type Arginase (Arginase I). No cross-reactivity has been observed for human Arginase II.

Among animal species, specific signal was observed in Rhesus monkey monkey serum. The signal was equivalent to 34 ng/ml of human arginase.

No signal has been obtained when sera of the following species were measured in the assay: rabbit, hamster, mouse, rat, dog, pig, sheep, goat, cow and horse.

- **Precision**

Intra-assay (Within-Run) (n=8)

Sample	Mean (ng/ml)	Standard Deviation (ng/ml)	CV (%)
1	8.75	0.59	6.7
2	28.74	1.41	4.9

Inter-assay (Run-to-Run) (n=8)

Sample	Mean	Standard Deviation	CV
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	(ng/ml)	(ng/ml)	(%)
1	8.83	0.76	8.6
2	29.65	2.16	7.3

- **Spiking Recovery**

Serum samples were spiked with different amounts of recombinant Arginase and assayed.

<i>Sample</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	8.69	-	-
	24.96	28.69	87
	40.89	48.69	84
	81.59	88.69	92
2	27.93	-	-
	42.66	47.93	89
	56.38	67.93	83
	90.66	107.93	84

- **Dilution Linearity**

Serum samples (4-times diluted) were further serially diluted with Dilution Buffer and assayed.

<i>Sample</i>	<i>Dilution</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	-	45.27	-	-
	1:2	19.78	22.64	87
	1:4	11.92	11.32	105

	1:8	4.78	5.66	85
2	-	81.77	-	-
	1:2	37.65	40.89	92
	1:4	18.22	20.44	89
	1:8	9.02	10.22	88

14. Troubleshooting and FAQs

1/ Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature

2/ High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time should be decreased before addition of Stop Solution

3/ High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing

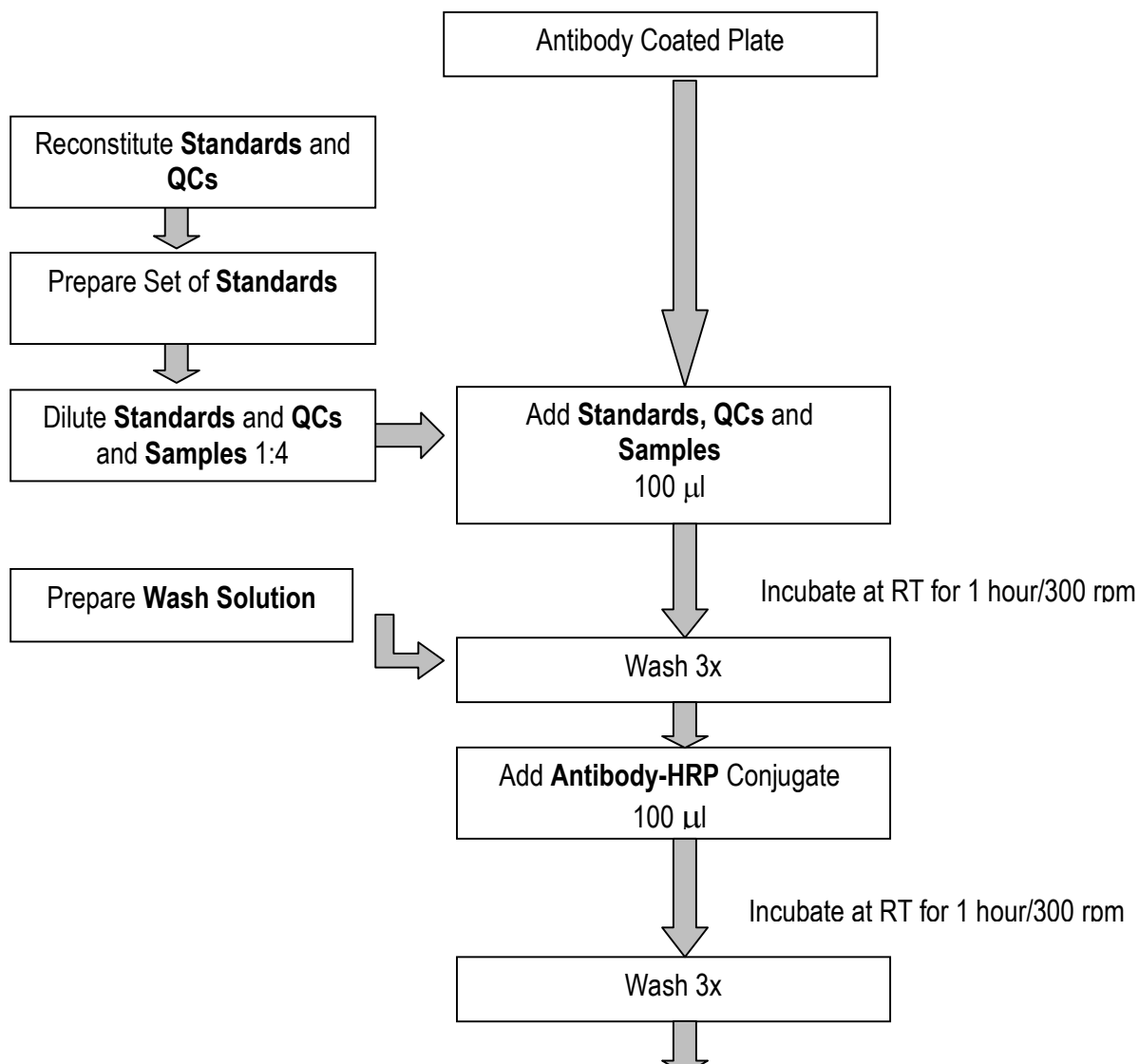
15. References

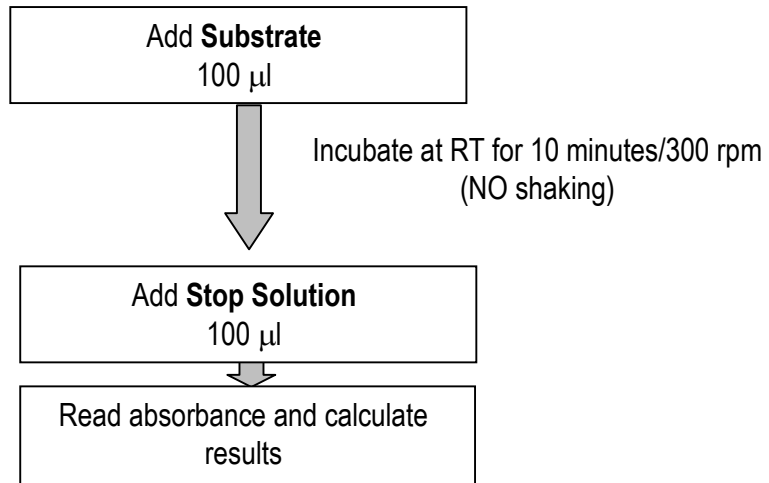
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Assay Procedure Summary





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Notes:
