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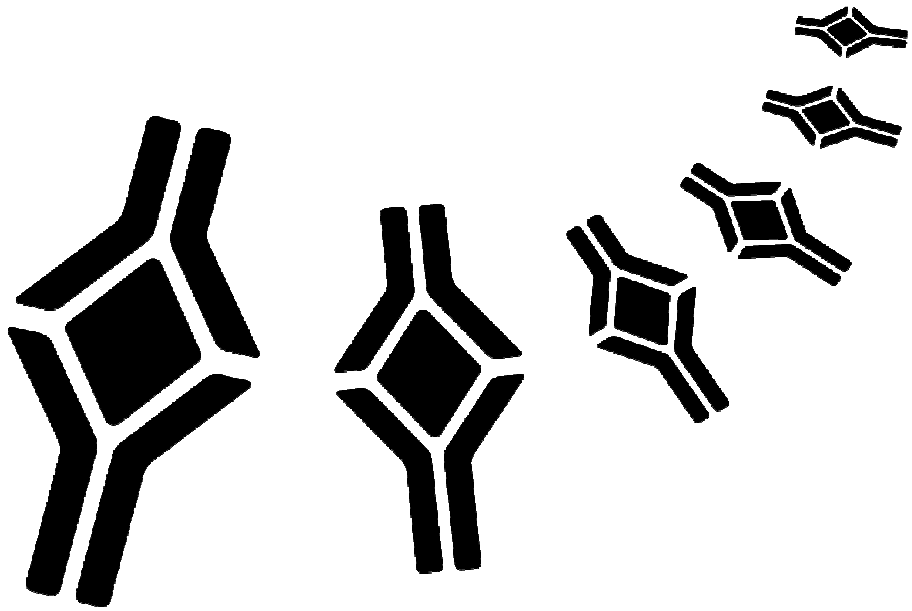
Research
and Diagnostic Products



HUMAN AMINOACYLASE-1 ELISA

Product Data Sheet

Cat. No.: RD192406200R

For Research Use Only

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorní medicína a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The RD192406200R Human Aminocylase-1 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human aminoacylase-1 (ACY-1).

Features

- **It is intended for research use only**
- The total assay time is less than 4.5 hours
- The kit measures total aminoacylase-1 in serum, plasma (citrate, heparin) and urine
- Assay format is 96 wells
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

Aminoacylase-1 (ACY-1, N-acyl-L-amino-acid amidohydrolase) belongs to the M20 family of metalloproteases. It is a 408-amino-acid protein with a molecular weight of 45.8 KDa which catalyzes the hydrolysis of N-acetylated amino acids into the free amino acid and acetic acid [1]. ACY-1 plays a general role in the cytosolic breakdown of acetylated amino acids generated during intracellular protein degradation. The gene encoding ACY-1 is evolutionarily conserved in fish, frog, mouse, rat and human.The expression of ACY-1 is highest in the kidney and brain.

Aminoacylase-1 is a potential biomarker of long-term outcome after kidney transplantation. Significant differences in serum ACY-1 levels were apparent between delayed, slow and immediate graft function. ACY-1 was not detected in the majority of patients before transplantation and remained low in recipients of live-donor kidneys. Serum ACY-1 levels were increased significantly in the majority of patients with delayed graft function (DGF), but rose in only a minority of patients with immediate function. These analyses provide novel insights into the potential clinical utility of serum ACY-1 levels immediately post kidney transplation, enabling subdivision of patients with delayed graft function in terms of long-term outcome. Serum ACY-1 concentration was high in patients with tubular cell damage due to ischemia-reperfusion injury (IRI) and/ or acute tubular necrosis [2,3].

ACY-1 also interacts with sfingosine kinase type 1, which is involved in promoting cell growth and inhibiting apoptosis of tumor cells. ACY-1 plays a role in regulating responses of the cell to oxidative stress [4,5]. ACY-1 serves as a putative suppressor in renal cell carcinoma and small cell lung cancer [6]. ACY-1 expression is also significantly correlated with serum alpha fetoprotein level and tumor invasiveness. It was also demonstrated that ACY-1 acts as a tumor suppresor in hepatocellular carcinoma [7].

An enzymatic deficiency in ACY-1 may result in defects of brain metabolism and function, such as encephalopathy, unspecific psychomotor delay, psychomotor delay with atrophy of the vermis and syringomyelia, marked muscular hypotonia or normal clinical features (e.g. fever). Epileptic seizures are a frequent feature in these patients. ACY-1 deficiency has also been described as an inborn error of metabolism. This disorder was identified in children who have increased urinary excretion of N-acetylamino acids [1].

<u>Areas of investigation:</u>
Renal disease
Transplantation
Neural and brain disease
Oncology

4. TEST PRINCIPLE

In the BioVendor Human Human Aminocylase-1 ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human aminoacylase-1 antibody. After 120 minutes incubation and a washing, biotin-labelled monoclonal anti-human aminoacylase-1 antibody is added and incubated with captured ACY-1 for 60 minutes. After another washing,streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining 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. The absorbance is proportional to the concentration of ACY-1. A standard curve is constructed by plotting absorbance values against concentrations of aminoacylase-1 standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit may contain components of human or animal origin. These materials should be handled as potentially infectious
- 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
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody	lyophilized	2 vials
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Dilution Buffer	ready to use	50 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 5 -1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

9. PREPARATION OF REAGENTS

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

Streptavidin-HRP Conjugate
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 Aminoacylase-1 Master Standard
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!
Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (do not foam).
The resulting concentration of ACY-1 in the stock solution is **30 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	–	30.0 ng/ml
250 µl of stock	250 µl	15.0 ng/ml
250 µl of 15.0 ng/ml	250 µl	7.50 ng/ml
250 µl of 7.50 ng/ml	250 µl	3.75 ng/ml
250 µl of 3.75 ng/ml	250 µl	1.88 ng/ml
250 µl of 1.88 ng/ml	250 µl	0.94 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
Do not store the reconstituted Master Standard and/or diluted standard solutions.

Biotin Labelled Antibody
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of Biotin Labelled Antibody!!!
Reconstitute the lyophilized Biotin Labelled Antibody with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Dilute Biotin Labelled Antibody Concentrate 100x with Dilution Buffer (e.g. 10 µl of Biotin Labelled Antibody Concentrate + 990 µl of Dilution Buffer for 8 wells).
Stability and storage:
Do not store the reconstituted and/or diluted Biotin Labelled Antibody solutions.

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution, e.g. 100 ml of Wash Solution Concentrate (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 Concentrate (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures human aminoacylase-1 in serum, plasma (citrate, heparin) and urine.

Samples can be assayed immediately after collection, or after long-term storage. Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

An appropriate dilution should be assessed by the researcher in advance to batch measurement.
Recommended starting dilution for serum and plasma is 10x and recommended starting dilution for urine is 3x.

Dilute serum samples 10x with Dilution Buffer just prior to the assay, e.g. 15 µl of sample + 135 µl of Dilution Buffer for singlets, or preferably 25 µl of sample + 225 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Dilute urine samples 3x with Dilution Buffer just prior to the assay, e.g. 40 µl of sample + 80 µl of Dilution Buffer for singlets, or preferably 80 µl of sample + 160 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°C, or preferably at -70°C or lower for long-term storage. Avoid repeated freeze/thaw cycles.
Do not store the diluted samples.

See Chapter 13 for effect of sample matrix (serum/plasma) on the concentration of human aminoacylase-1.

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

11. ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **2 hours**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
4. Pipet **100 µl** of Biotin Labelled Antibody 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 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
7. Pipet **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against a paper towel.
10. 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.
11. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 30 minutes] if the reaction temperature is less than 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 12.**

Note 1: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine aminoacylase-1 concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat four times. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 30**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	**Standard 15**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	**Standard 7.5**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	**Standard 3.75**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	**Standard 1.88**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	**Standard 0.94**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
G	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40
H	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The Standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of aminoacylase-1 (ng/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve (i.e. *logit* of absorbance (Y) is plotted against *log* of the known concentration (X) of standards).

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay; e.g. 2 ng/ml (from standard curve) x 10 (dilution factor) = 20 ng/ml.

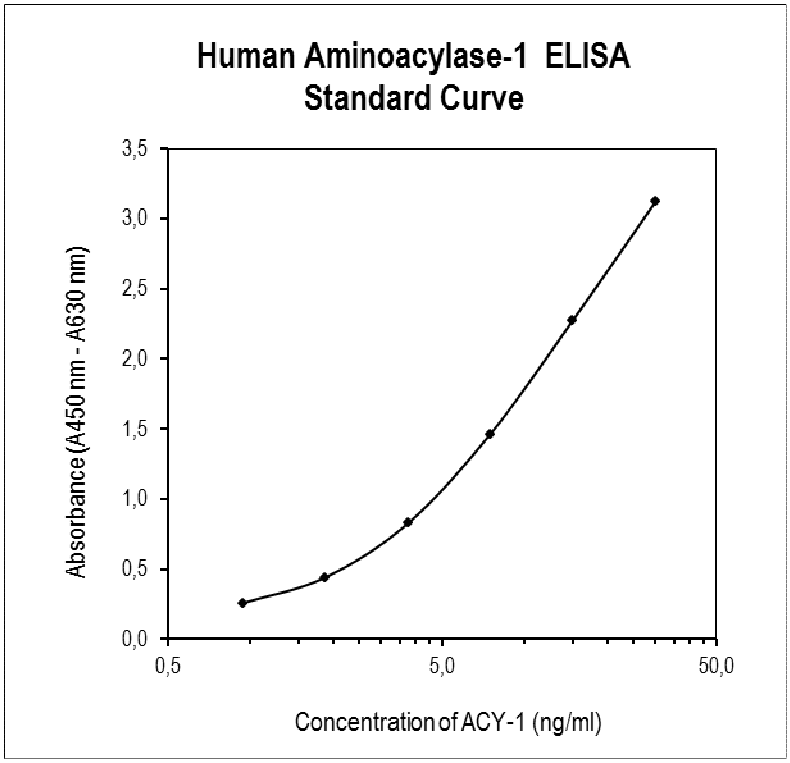


Figure 2: Typical Standard Curve for Human Aminoacylase-1 ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human Aminoacylase-1 ELISA are presented in this chapter

- **Sensitivity**

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + $3xSD_{blank}$) is calculated from the real aminoacylase-1 values in wells and is 0.14 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

- **Specificity**

The antibodies used in this ELISA are specific for human aminoacylase-1.

» Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	38.0	2.35	6.2
2	86.4	5.47	6.3

Inter-assay (Run-to-Run) (n=5)

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	19.7	1.32	6.7
2	61.8	5.24	8.5

- **Spiking Recovery**

Serum samples were spiked with different amounts of human aminoacylase-1 and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	22.2 32.5 41.3 61.8	- 31.6 41.0 59.7	- 102.9 100.7 103.4
2	19.0 29.1 38.3 54.0	- 28.4 37.8 56.5	- 102.4 101.4 95.6

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml))*	*Recovery **O/E** (%)*
1	- 2x 4x 8x	93.8 46.0 23.7 11.7	- 46.9 23.5 11.7	- 98.1 100.9 99.9
2	- 2x 4x 8x	140.4 69.4 35.6 17.7	- 70.2 35.1 17.6	- 98.9 101.5 101.1

- **Effect of sample matrix**

EDTA, citrate and heparin plasma samples were compared to respective serum samples from the same 10 individuals. Results are shown below:

Volunteer No.	*Serum (ng/ml)*	*Plasma (ng/ml)*		
		EDTA	*Citrate*	*Heparin*
1	182,6	61.8	147. 9	187.5
2	87.8	25.4	78.1	96.3
3	28.1	12.7	23.4	26.5
4	14.5	5.18	14.0	13.0
5	21.1	9.74	17.2	22.3
6	34.2	11.8	37.6	48.6
7	26.4	12.1	32.6	34.9
8	27.5	7.63	25.9	32.3
9	46.6	12.5	40.2	50.0
10	28.2	8.87	25.1	32.0
Mean (ng/ml)	**49.6**	**16.8**	**44.2**	**54.3**
Mean Plasma/Serum (%)		**33.8%**	**89.1%**	**109.6%**
Coefficient of determination R^2		**0.98**	**0.99**	**0.99**

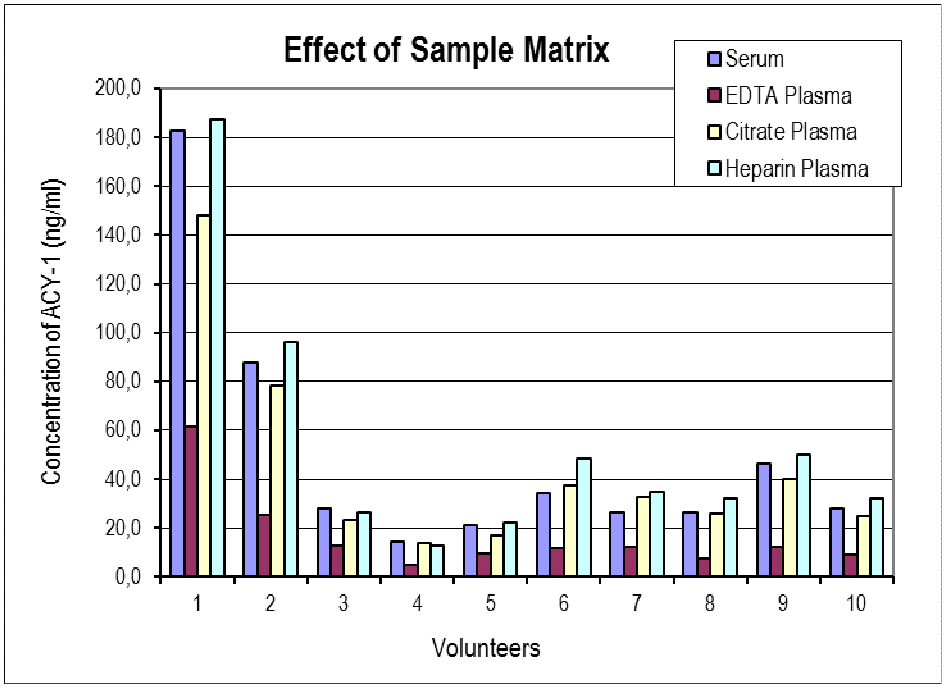


Figure 3: Aminoacylase-1 levels measured using Human Aminoacylase-1 ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

14. DEFINITION OF THE STANDARD

The Standard used in this kit is recombinant protein. The recombinant human aminoacylase-1, produced in Spodoptera frugiperda, is 47 kDa protein consisting of 407 amino-acid residues of human aminoacylase-1 (Thr2-Ser408) and 10 additional amino acids.

15. PRELIMINARY POPULATION DATA

The following results were obtained when serum samples from 155 unselected donors (89 men + 66 women) 20–65 years old were assayed with the BioVendor Human Aminoacylase-1 ELISA in our laboratory.

Sex	*Age (years)*	*n*	Aminoacylase-1 *(ng/ml)*				
			Mean	*Median*	*SD*	*Min*	*Max*
Men	20-29	18	49.1	47.7	25.3	16.2	122.4
	30-39	26	85.7	42.5	87.3	21.0	304.7
	40-49	31	73.0	58.3	42.1	29.2	208.0
	50-65	14	76.5	48.9	72.7	28.8	314.8
Women	20-29	12	33.3	30.3	20.5	15.2	96.5
	30-39	26	42.8	27.6	41.2	9.94	188.2
	40-49	20	49.1	28.0	52.5	15.6	253.7
	50-61	8	87.4	55.2	87.5	31.5	304.8

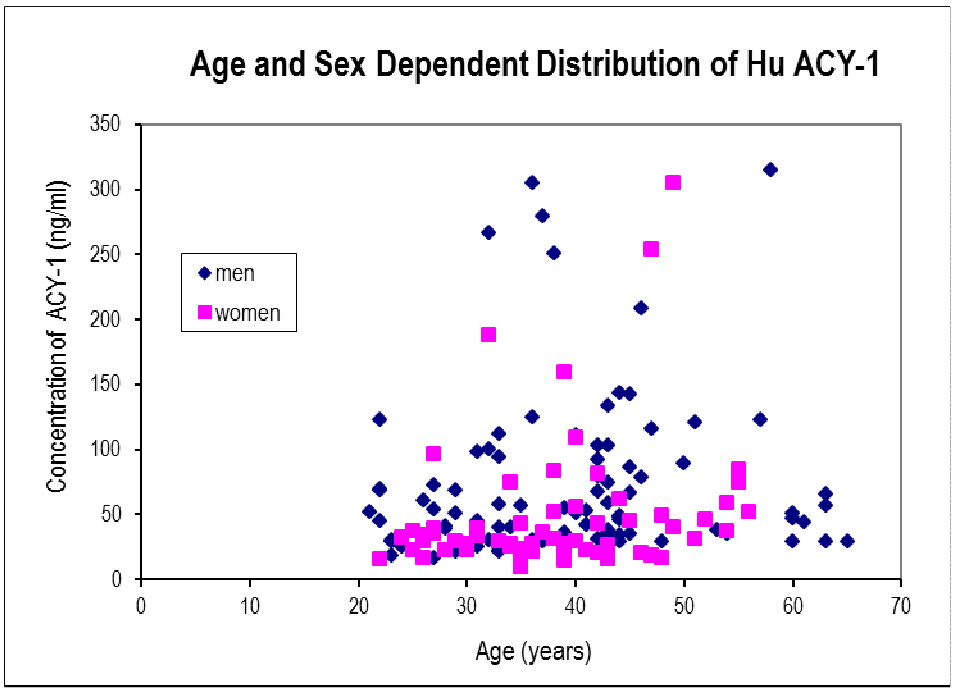


Figure 4: Human aminoacylase-1 concentration plotted against donor age and sex.

- **Reference range**

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for aminoacylase-1 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Aminoacylase-1 ELISA has not been compared to any commercial immunoassay.

17. TROUBLESHOOTING AND FAQS

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
- Manual washing
- Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards or samples

18. REFERENCES

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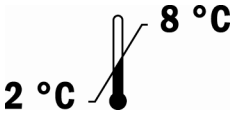
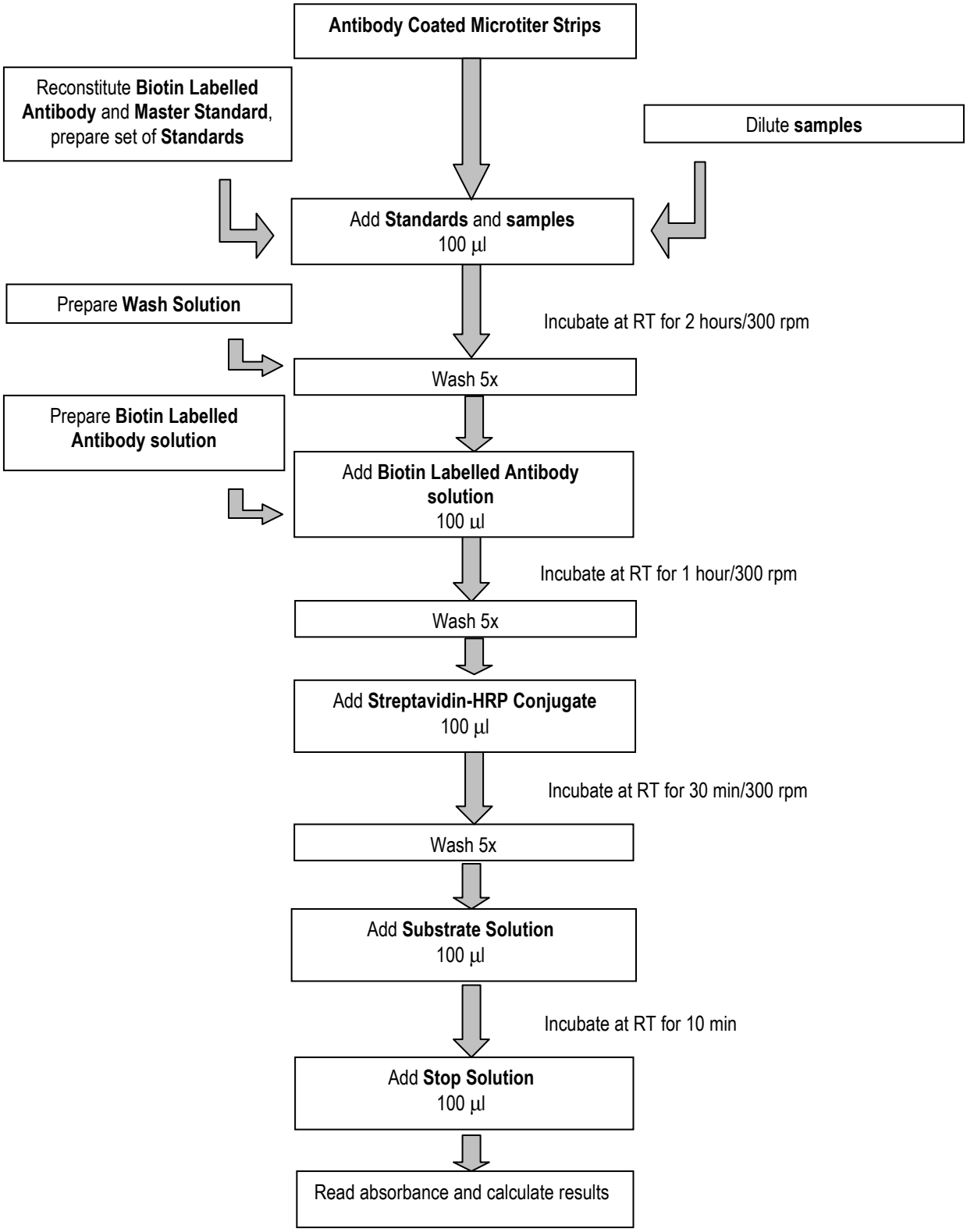
9. Baumeier Ch, Kaiser D, Heeren J, Scheja L, John C, Weise Ch, Eravci M, Lagerpusch M, Schulze G, Joost HG, Schwenk RW, Schürman A: Caloric restriction and intermittent fasting alter hepatic lipid droplet proteome and diacylglycerol species and prevent diabetes in NZO mice. Biochimica and Biophysica Acta 2015; 1851: 566-576.

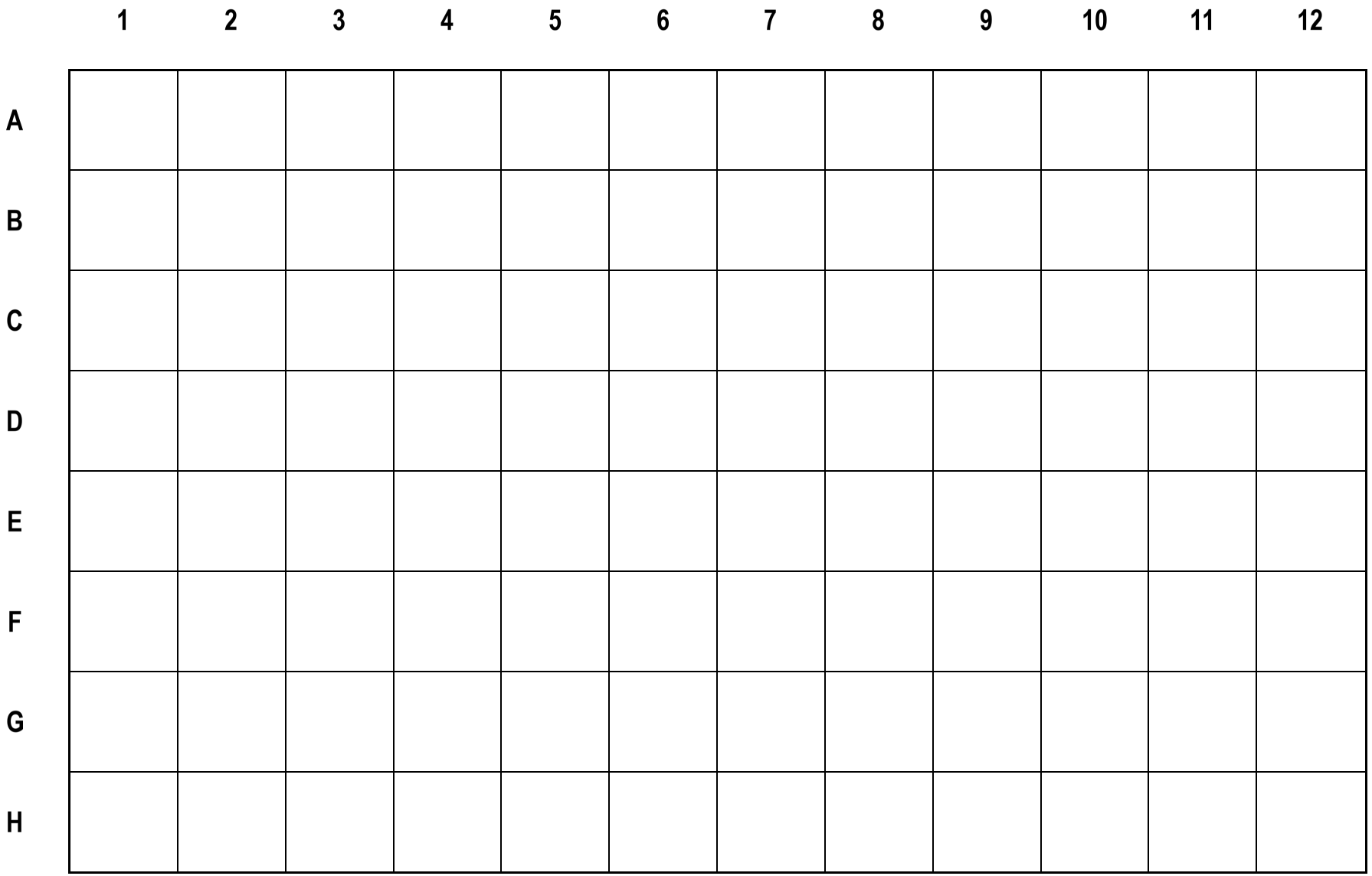
» For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

Assay Procedure Summary

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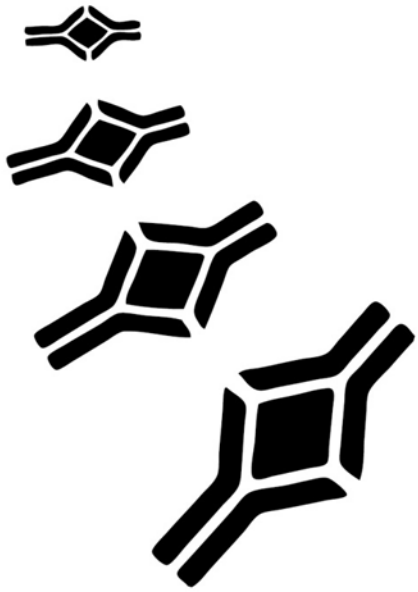

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