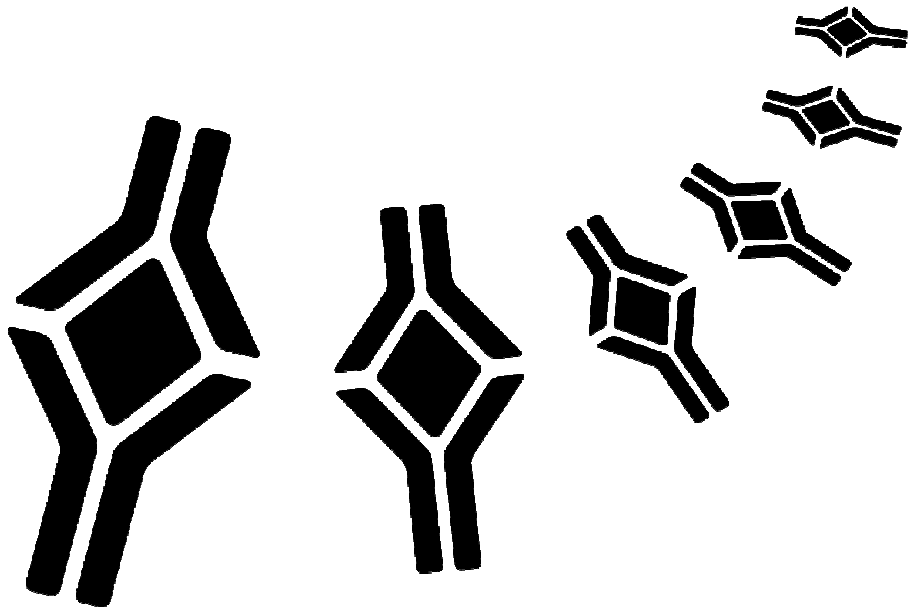
BioVendor
Research
and Diagnostic Products



HUMAN APOLIPOPROTEIN D ELISA

Product Data Sheet

Cat. No.: RD193118200R

For Research Use Only

VERSION 21 121211 45

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 RD193118200R Human Apolipoprotein D ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Apolipoprotein D.

Features

- **For research use only!**
- The total assay time is less than 4 hours
- The kit measures total Apolipoprotein D in serum, plasma (EDTA, citrate, heparin), cerebrospinal fluid and urine
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is recombinant protein
- 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

Apolipoprotein D, a glycoprotein with MW of approximately 29 kDa, is associated with HDL in plasma. Though it is called apolipoprotein, it is more similar to lipocalins. The mature protein consists of 169 amino-acids, 5 of which are cysteines responsible for intra-molecular bonds as well as disulfide bonding with Apo A-II) through which ApoD is peripherally associated with HDL (especially in HDL_3). In contrast to other apolipoproteins ApoD consists mainly of β-sheets. As with other lipocalins, the tertiary structure creates a β-barrel, the cavity of which is the binding place for small hydrophobic molecules (e.g. cholesterol, progesterone, bilirubin and arachidonic acid).

Unlike other apolipoproteins, ApoD is poorly expressed in the liver and intestine in humans. Higher expression was observed in adrenal glands, pancreas, kidneys, placenta, spleen, lungs, ovaries, testes, brain (astrocytes), peripheral nerves and cerebrospinal fluid.

The exact function of ApoD is yet unknown. There are several hypotheses more or less supported by evidence. One hypothesis derived from overexpression studies in mice claims that an over-expression of ApoD driven by neuronal promoters amplifies the insulin resistance that occurs with aging. These mice are not obese and present normal food intake and normal lipid levels in circulation, but they develop glucose intolerance and insulin resistance with aging.

ApoD interacts with the cytoplasmic portion of the long form of the leptin receptor, thus it may play a role in the control of food intake and body weight. ApoD may also be involved in triglyceride metabolism. Three distinct mutations of the ApoD gene have been identified and each of them is associated with significantly elevated plasma triglyceride levels and reduced HDL-cholesterol levels, a plasma lipid profile that is characteristic of metabolic syndrome.

ApoD is also shown to modulate the activity of lecithin:cholesterol acyltransferase (LCAT), an HDL-bound enzyme which catalyzes the conversion of free cholesterol ester which is subsequently recruited into the core of HDL. However, it is not yet clear whether apoD activates or inhibits LCAT activity.

Nieman-Pick (NPC) disease is a human neurodegenerative disorder characterized by impaired intracellular cholesterol transport. In rodent models of NPC disease, ApoD protein levels are markedly elevated by 30-fold in the brain and 6-fold in plasma, correlating with increased intracellular cholesterol storage.

ApoD may play an important role in oxidative stress. Do Carmo et al. show that ApoD expression is significantly induced in response to cellular stress regardless of whether the stress condition is caused by lipopolysaccharide (LPS) stimulation, H_2O_2 treatment or UV-light irradiation. ApoD also confers a neuroprotective effect in the brain of mice.

<u>Areas of investigation:</u>
Energy metabolism and body weight regulation
Neural tissue damage markers
Lipoprotein metabolism
Cardiovascular disease

4. TEST PRINCIPLE

In the BioVendor Human Apolipoprotein D ELISA, standards, quality controls and samples are incubated in microtitrate plate wells pre-coated with polyclonal anti-human Apolipoprotein D antibody. After 120 min incubation and a washing, biotin-labelled polyclonal anti-human ApoD antibody is added and incubated with captured ApoD for 60 min. After another washing, the streptavidin-HRP conjugate is added. After 30 min 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 Apolipoprotein D. A standard curve is constructed by plotting absorbance values against concentrations of Apolipoprotein D 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 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
- This kit contains components of 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 Conc. (100x)	concentrated	0.13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	1 vial
Quality Control HIGH	lyophilized	1 vial
Quality Control LOW	lyophilized	1 vial
Biotin-Ab Diluent	ready to use	13 ml
Dilution Buffer	ready to use	65 ml
Wash Solution Conc. (10x)	concentrate	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 microtitrate 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 desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate
Biotin-Ab 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 Apolipoprotein D 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 (not to foam). The resulting concentration of ApoD in the stock solution is **10 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	10 ng/ml
250 µl of std. 10 ng/ml	250 µl	5 ng/ml
250 µl of std. 5 ng/ml	250 µl	2.5 ng/ml
250 µl of std. 2.5 ng/ml	250 µl	1.25 ng/ml
250 µl of std. 1.25 ng/ml	250 µl	0.63 ng/ml
250 µl of std. 0.63 ng/ml	250 µl	0.31 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
Standard stock solution (10 ng/ml) must be used immediately or aliquoted and frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.
Do not store the diluted standard solutions.

Quality Controls HIGH, LOW
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Controls concentrations!!!
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Dilute Quality Controls prior to use 5000x with Dilution Buffer in two steps as follows:
Dilution A (50x):
Add 5 µl of Quality Control into 245 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.
Dilution B (100x):
Add 5 µl of Dilution A into 495 µl of Dilution Buffer to prepare final dilution (5 000x). It means the final dilution is 5000x and the concentration of Quality Control calculated from the standard curve must be multiplied by a dilution factor of 5000.
Mix well (not to foam). Vortex is recommended. Beware of imprecision in pipetting.
Note:
Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

Stability and storage:
Reconstituted Quality Controls should be aliquoted and frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.
Do not store the diluted Quality Controls.

Biotin Labelled Antibody Conc. (100x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) to 99 parts Biotin-Ab Diluent. Example: 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Biotin-Ab Diluent for 1 strip (8 wells).
Stability and storage:
Opened Biotin Labelled Antibody Concentrate (10x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Biotin Labelled Antibody solution.

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in 900 ml of 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 Apolipoprotein D in serum, plasma (EDTA, citrate, heparin) and urine.
Samples should be assayed immediately after collection or should be stored at -20°C. 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.

Serum and plasma samples:
Dilute serum or plasma samples 5 000x with Dilution Buffer just prior to the assay in two steps as follows:
Dilution A (50x):
Add 5 µl of sample into 245 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.
Dilution B (100x):
Add 5 µl of Dilution A into 495 µl of Dilution Buffer to prepare final dilution (5 000x). **Mix well** (not to foam). Vortex is recommended.
CSF and urine samples:
Dilute urine samples just prior to the assay 125x with Dilution Buffer, e.g. 5 µl of sample + 620 µl Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

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

See Chapter 13 for stability of serum and plasma samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of Apolipoprotein D.

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, Quality Controls, 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 paper towel.
4. Pipet **100 µl** of Biotin Labelled Antibody 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. Incubation without shaking is the alternative that requires to extent incubation with substrate – see point 11.
6. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against 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 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 20 minutes] if the reaction temperature is below than 20°C. Do not shake with 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 9.**

Note: 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 Apolipoprotein D 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: Remove liquid from wells completely and pipet 0.35 ml Wash Solution into each well. ***Allow Wash Solution to stay in each well for 30 seconds.*** *Remove solution from wells completely and repeat washing four times. After final wash, invert and tap the plate strongly against paper towel.* ***Make certain that liquid has been removed from wells entirely after each washing step.***

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 10**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 5**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 2.5**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 1.25**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 0.63**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 0.31**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**QC HIGH**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC LOW**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

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 Apolipoprotein D (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. 3 ng/ml (from standard curve) x 5000 (dilution factor) = 15000 ng/ml = 15 µg/ml.

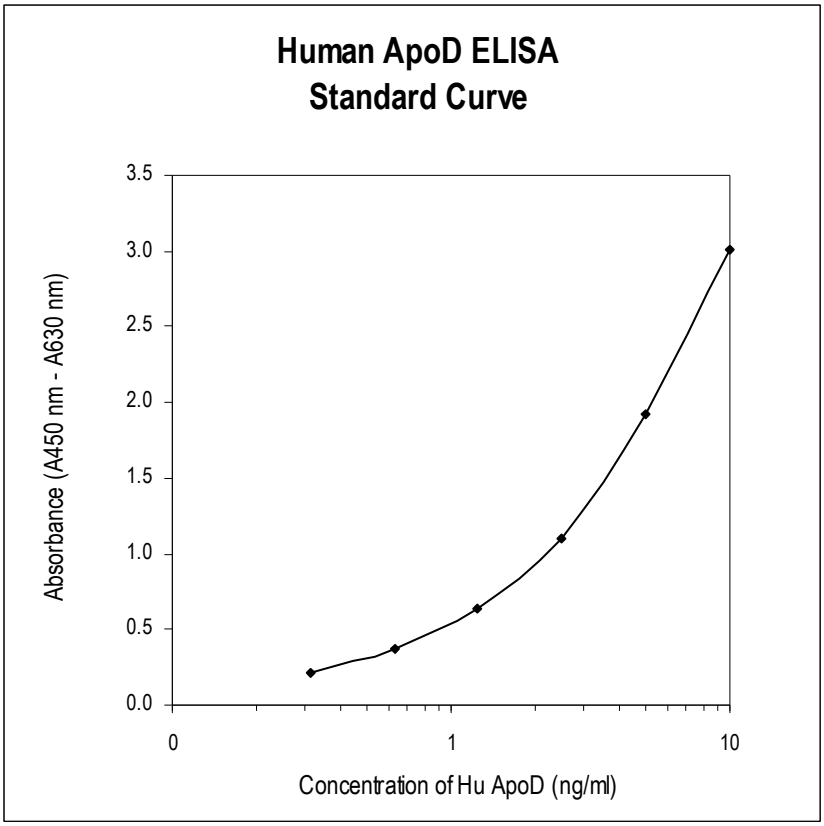


Figure 2: Typical Standard Curve for Human Apolipoprotein D ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Apolipoprotein D 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 Apolipoprotein D values in wells and is 0.086 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding Apolipoprotein D level of 10 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the Apolipoprotein D concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human Apolipoprotein D.

Sera of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com.

Mammalian serum sample	*Observed crossreactivity*
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	no
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

» **Presented results are multiplied by respective dilution factor**

- **Precision**

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

Sample	*Mean (µg/ml)*	*SD (µg/ml)*	*CV (%)*
1	7.59	0.26	3.61
2	4.63	0.08	1.86

Inter-assay (Run-to-Run) (n=6)

Sample	*Mean (µg/ml)*	*SD (µg/ml)*	*CV (%)*
1	2.09	0.14	6.92
2	4.71	0.35	7.45

- **Spiking Recovery**

Serum samples were spiked with different amounts of human Apolipoprotein D, diluted with Dilution Buffer 5000x and assayed.

Sample	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	3.47 4.72 9.15 27.63	- 5.02 9.72 28.47	- 94 94 97
2	4.69 5.85 10.85 28.98	- 6.24 10.94 29.69	- 94 99 98

- **Linearity**

Serum samples were serially diluted with Dilution Buffer after primary dilution 5000x and assayed.

Sample	*Dilution*	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	-	11.23	-	-
	2x	6.02	5.61	107
	4x	3.25	2.81	116
	8x	1.36	1.40	97
2	-	8.12	-	-
	2x	4.02	4.06	99
	4x	2.19	2.03	108
	8x	1.07	1.01	106

- **Effect of sample matrix**

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals.

Results are shown below:

Volunteer No.	*Serum (µg/ml)*	*Plasma (µg/ml)*		
		EDTA	*Citrate*	*Heparin*
1	4.86	5.84	4.22	6.14
2	4.50	4.90	3.68	5.18
3	5.12	5.79	4.26	6.20
4	6.06	6.15	4.83	6.86
5	3.02	3.32	2.73	3.50
6	2.64	3.15	2.25	3.48
7	2.01	2.27	1.75	2.58
8	2.96	2.91	2.33	3.05
9	4.83	5.80	5.00	6.63
10	4.94	5.73	4.57	6.11
Mean (ng/ml)	**4.09**	**4.59**	**3.56**	**4.97**
Mean Plasma/Serum (%)	**-**	**112**	**87**	**121**
Coefficient of determination R^2	**-**	**0.97**	**0.97**	**0.97**

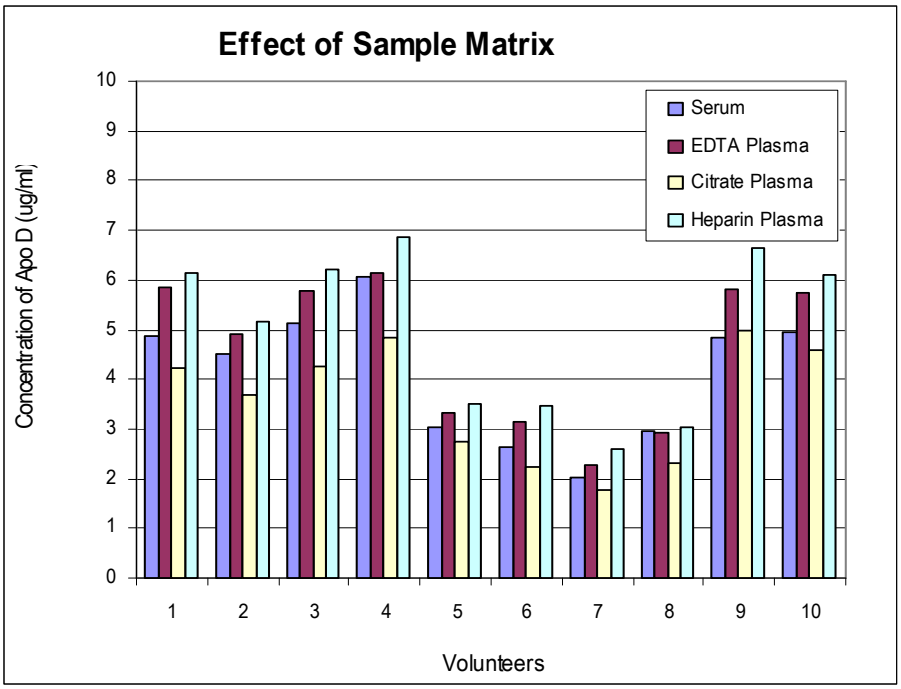


Figure 3: Apolipoprotein D levels measured using Human Apolipoprotein D ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- **Stability of samples stored at 2-8°C**

Samples should be stored at -20°C. However, no decline in concentration of Apolipoprotein D was observed in serum and plasma samples after 14 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	*Incubation Temp, Period*	*Serum (µg/ml)*	*Plasma (µg/ml)*		
			EDTA	*Citrate*	*Heparin*
1	-20°C	5.75	5.22	5.84	6.07
	2-8°C, 1 day	5.89	4.76	6.15	5.72
	2-8°C, 7 days	5.60	4.74	5.91	5.94
2	-20°C	3.63	3.87	4.40	4.08
	2-8°C, 1 day	3.68	3.67	4.04	3.99
	2-8°C, 7 days	3.73	3.71	4.19	4.15
3	-20°C	6.54	6.31	8.21	7.57
	2-8°C, 1 day	6.87	6.16	7.32	7.73
	2-8°C, 7 days	6.64	6.51	7.77	7.85

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human Apolipoprotein D in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	*Number of f/t cycles*	*Serum (µg/ml)*	*Plasma (µg/ml)*		
			EDTA	*Citrate*	*Heparin*
1	1x	3.85	3.79	3.35	3.69
	3x	3.76	4.35	3.49	4.31
	5x	3.92	4.10	3.51	3.60
2	1x	7.11	6.73	6.20	7.82
	3x	7.05	6.94	6.23	6.80
	5x	6.98	7.13	6.31	6.99
3	1x	7.40	7.38	6.75	7.85
	3x	7.74	7.97	7.35	7.71
	5x	7.31	7.92	6.89	7.75

14. DEFINITION OF THE STANDARD

The Standard used in this kit is recombinant protein. Recombinant human ApoD, produced on E. Coli, is 19.82 kDa protein consisting of 174 amino-acid residues of human ApoD and 7 additional amino-acids.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 168 unselected donors (91 men + 77 women) 20 - 69 years old were assayed with the Biovendor Human Apolipoprotein D ELISA in our laboratory:

- **Age and Sex dependent distribution of Apolipoprotein D**

Sex	Age (years)	n	Mean	SD	Min	Max
			ApoD (µg/ml)			
Men	20-39	48	5.08	1.39	2.67	8.39
	40-69	43	5.35	1.68	2.35	10.49
Women	20-39	43	4.49	1.55	1.04	8.51
	40-69	34	4.49	1.29	2.14	7.51

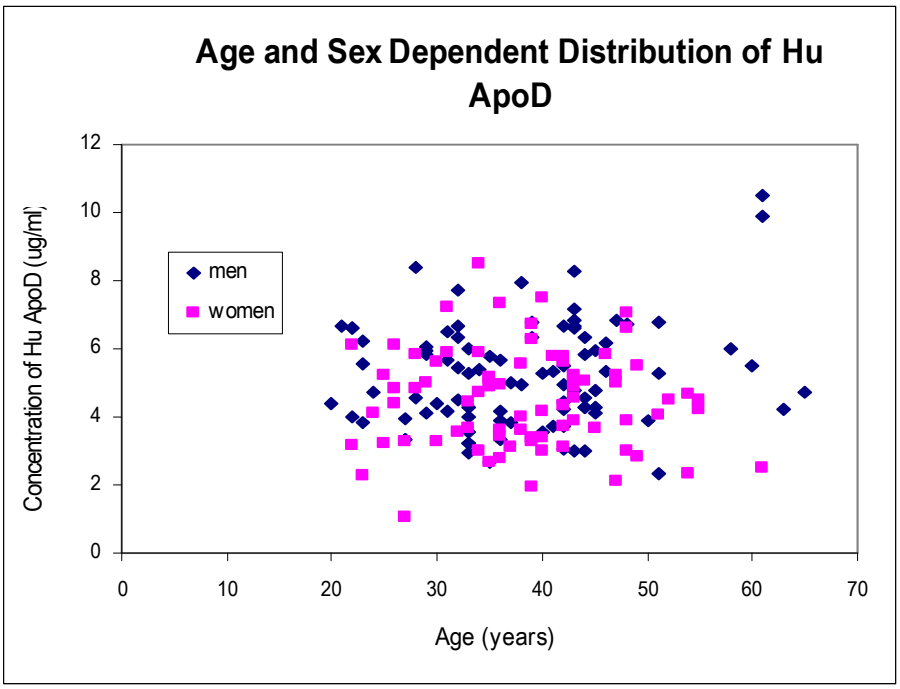


Figure 4: Human Apolipoprotein D concentration plotted against donor age and sex.

- **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 references ranges for ApoD levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Apolipoprotein D 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
- 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, Quality Controls or samples

18. REFERENCES

References to Apolipoprotein D:

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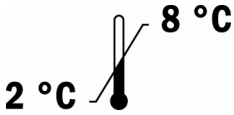
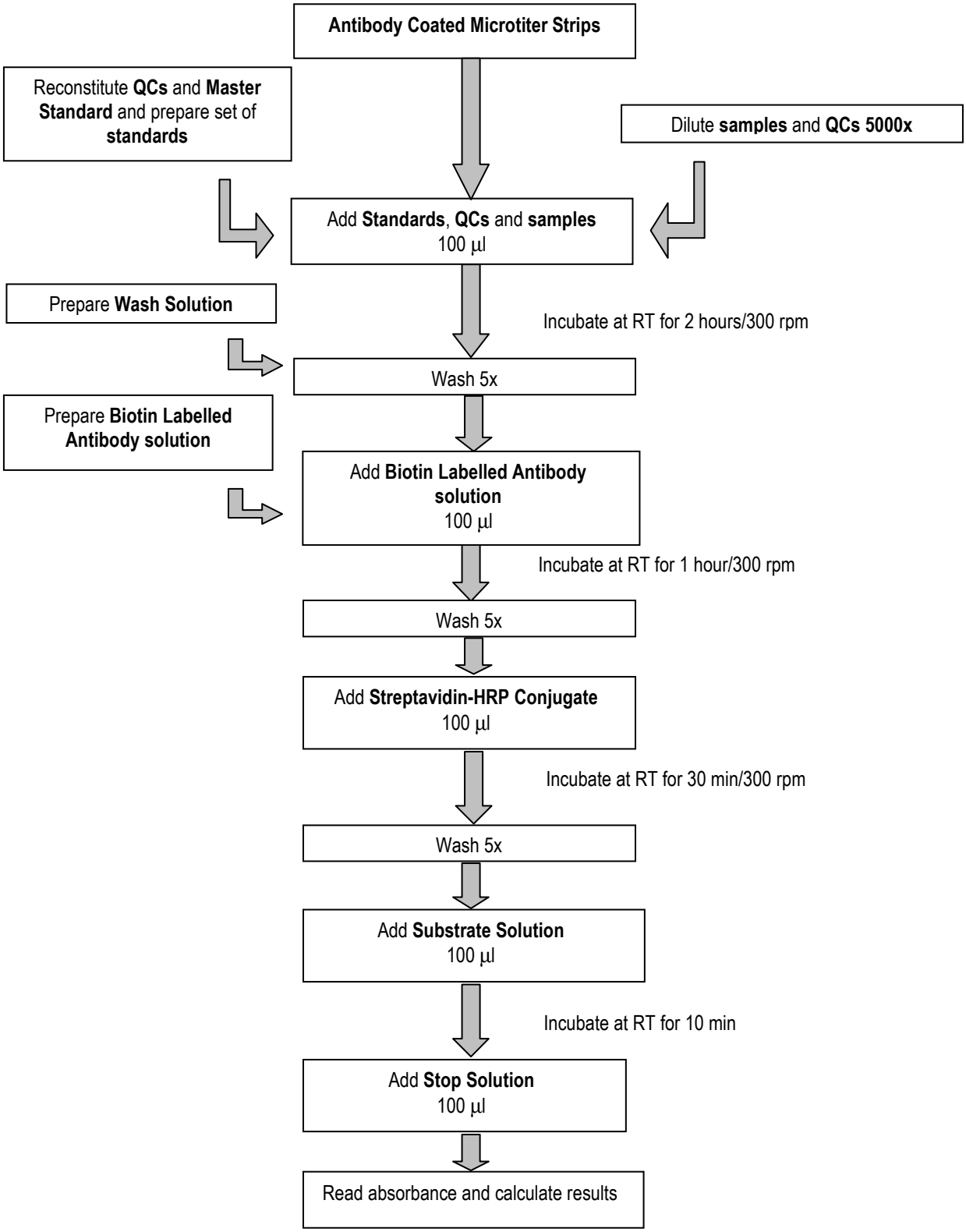
- Do Carmo, S., Fournier D., Mournier C., Rassart E.: Human Apolipoprotein D over-expression in transgenic mice induces insulin resistance and alters lipid metabolism. Am J Physiol Edocrinol Metab 2009 296:802 – 811
- Perdomo, G., Dong, H. H.: Apolipoprotein D in lipid metabolism and its functional implication in atherosclerosis and aging. Aging 2009; 1(1): 17 - 27
- Eichinger, A, Nasreen A., Kim H. J., Skerra A.: Structural insight into the dual ligand specifity and mode of high density lipoprotein association of apolipoprotein D. J Biol Chem 2007; 282 (42): 31068 - 31075
- Do Carmo, S. et al.: Modulation of Apolipoprotein D levels in human pregnancy and association with gestational weight gain. Rep Biol Endocrinol 2009; 282 (42): 31068 - 31075

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



	A	B	C	D	E	F	G	H
12								
11								
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Research
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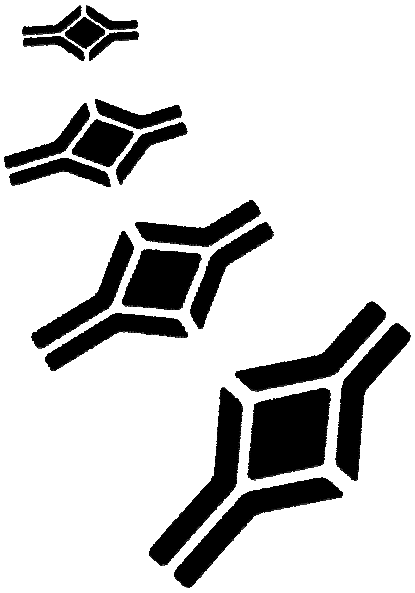

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