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BioVendor
Research
and Diagnostic Products

Human Adiponectin ELISA

Cat. No.: RD195023100R

Manufacturer

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CONTENTS:

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

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1. Intended Use

The RD195023100R Human Adiponectin ELISA is a competitive Enzyme-linked immunosorbent Assay for the quantitative measurement of human adiponectin in serum and plasma. It is intended for *in vitro* research use only.

Features

- The total assay time is less than three hours.
- The kit measures total serum or plasma adiponectin.
- Standards are human serum based.
- Quality controls are human serum based. No animal sera are used.
- Components of the kit are ready-to-use (with the exception of Wash Solution).

2. Storage, Expiration

Store the kit at 2-8°C. Under these conditions, assay components are stable till the expiry date is over. (See the expiry date indicated on the kit label).

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3. Summary

Adiponectin, also referred to as Acrp30, AdipoQ and GBP-28, is a recently discovered 244 aminoacid protein, the product of the *apM1* gene, which is physiologically active and specifically and highly expressed in adipose cells. The protein belongs to the soluble defence collagen superfamily; it has a collagen-like domain structurally homologous with collagen VIII and X and complement factor C1q-like globular domain. Adiponectin forms homotrimers, which are the building blocks for higher order complexes found circulating in serum. Together, these complexes make up approximately 0.01% of total serum protein. Adiponectin receptors AdipoR1 and AdipoR2 have been recently cloned; AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver.

Paradoxically, adipose tissue-expressed adiponectin levels are inversely related to the degree of adiposity. Adiponectin concentrations correlate negatively with glucose, insulin, triglyceride concentrations, liver fat content and body mass index and positively with high-density lipoprotein-cholesterol levels, hepatic insulin sensitivity and insulin-stimulated glucose disposal. Adiponectin has been shown to increase insulin sensitivity and decrease plasma glucose by increasing tissue fat oxidation. Of particular interest is that low adiponectin serum levels predict type 2 diabetes independent of other risk factors. Adiponectin also inhibits the inflammatory processes of atherosclerosis suppressing the expression of adhesion and cytokine molecules in vascular endothelial cells and macrophages, respectively. This adipokine plays a role as a scaffold of newly formed collagen in myocardial remodelling after ischaemic injury and also stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signalling in endothelial cells. Low serum adiponectin levels are found in patients with coronary artery disease.

Moreover, high circulating levels of adiponectin are associated with decreased risk of myocardial infarction, independent of other factors.

Altogether, adiponectin has the potential to become a clinically relevant parameter to be measured routinely in subjects at risk for type 2 diabetes, atherosclerosis and the metabolic syndrome.

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4. Test Principle

In the BioVendor's Human Adiponectin ELISA, standards, quality control and samples of sera (or plasma) are incubated in microtitration wells coated with recombinant human adiponectin together with horse radish peroxidase-labelled anti-adiponectin antibody (conjugate). After a thorough wash, the conjugate bound to the adiponectin coated wells is allowed to react with the hydrogen peroxide/TMB substrate solution. The reaction is stopped by addition of sulfuric acid solution and absorbance of the resulting yellow colour product is measured spectrophotometrically at 450 nm. The absorbance is inversely proportional to the adiponectin concentration. A standard curve is constructed by plotting absorbance values versus concentrations of serum adiponectin standards, and the concentrations of unknown samples are determined (µg/ml) using this standard curve.

5. Precautions

- For *in vitro* 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 of human origin.
- 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 specified on the kit label.

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6. Reagents Supplied

Cat. No.	*Kit Components*	*Quantity*
C111521	Microtiter Strips, coated with recombinant human Adiponectin, vacuum sealed	96 wells
C112112	Conjugate Solution (Anti-Adiponectin Antibody, Horseradish Peroxidase Conjugate), ready to use	7 ml
C113112	Human Adiponectin Standards (0.1, 0.2, 0.5, 1, 2, 5 and 10 µg/ml)	0.22 ml/vial
C114113	Quality Control High (RTU)	0.4 ml
C114213	Quality Control Low (RTU)	0.4ml
C005111	Dilution Buffer, ready to use	2 x 13 ml
C006121	Wash Solution Concentrate (10x)	100 ml
C007111	Substrate Solution (TMB), ready to use	2 x 13 ml
C008122	Stop Solution (0.5 M H_2SO_4), ready to use	9ml
-	Product Data Sheet + Certificate of Analysis	1 pc

7. Materials Required but Not Supplied

- Test tubes for diluting samples
- Precision pipettes to deliver 10-1000 µl and disposable tips
- Multichannel pipette 50-200 µl and disposable tips
- Microplate reader with 450 ± 10 nm filter
- Software package facilitating data generation and analysis
- Orbital microplate shaker capable of 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

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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 Wash Solution Concentrate.

Standards:
Dilute Standards 1:3 with Dilution Buffer prior to use (preferably 50 µl sample + 100 µl Dilution Buffer for duplicates).

Quality Controls:
Quality Controls are ready-to-use (RTU), supplied diluted 1:30. **Do not dilute prior the use.**

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.

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

9. Preparation of Samples

Dilute serum or plasma samples and 1:30 with Dilution Buffer (preferably 10 µl sample + 290 µl Dilution Buffer for duplicates).

Stability and storage:
Serum or plasma samples should be stored frozen (preferably at –80 °C, then the stability is at least 1 year). It has been proved that serum or plasma samples keep the adiponectin concentration for three thawing-freezing cycles, nevertheless, repeated thawing-freezing should be avoided.
Undiluted samples are stable at least 2 weeks at 2-8°C or 1 day at RT.
Diluted samples have to be stored frozen.

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10. Assay Procedure

1) Pipet 50 µl of diluted Standards, samples and **Quality Controls** (supplied ready-to-use, **do not dilute**), preferably in duplicates, into the appropriate wells.
2) Add 50 µl of Conjugate Solution.
3) Incubate the plate for 2 hours, shaking at ca. 300 rpm on an orbital microplate shaker.
4) Wash the wells 3-times with Wash Solution (0.35 ml per well).
5) Add 200 µl of Substrate Solution. (Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.)
6) Incubate the plate for 10-15 minutes at room temperature. (20-30°C).
7) Stop the colour development by adding 50 µl of Stop Solution.
8) Determine the absorbance by reading the plate at 450 nm. (The absorbance should be read within 5 minutes following step 7).

Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the lowest standard (the highest absorbance of the calibration curve), perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine Adiponectin concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

	strip 1+ 2	strip 3 + 4	strip 5+ 6	strip 7+ 8	strip 9+10	strip 11+ 12
A	Calibrator 10	QC High	Sample 7	Sample 15	Sample 23	Sample 31
B	Calibrator 5.0	QC Low	Sample 8	Sample 16	Sample 24	Sample 32
C	Calibrator 2.0	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	Calibrator 1.0	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	Calibrator 0.5	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	Calibrator 0.2	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	Calibrator 0.1	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

Figure 1: Example of work sheet.

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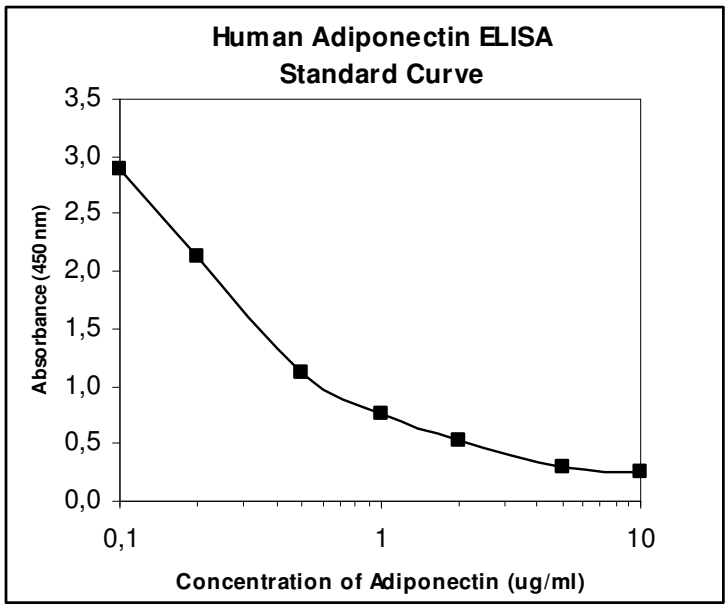
11. Calculations

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 Adiponectin (µg/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).
As the standards are to be diluted 3-times, while the samples and controls 30-times, the values calculated from the calibration curve have to be multiplied by a dilution factor of 10 to obtain the true results!



12. Limits of Assay

Results exceeding 100 µg/ml should be repeated with more diluted samples (e.g. 1:60). Dilution factors need to be taken into consideration in calculating the concentration of Adiponectin.

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13. Performance Characteristics

Typical analytical data of BioVendor Human Adiponectin 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 adiponectin concentration giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is defined as follows:

Analytical Limit of Detection is calculated from the real human adiponectin values in wells and is 7ng/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 = 7ng/ml x 30 = 210ng/ml

*Dilution Buffer is pipetted into blank wells.

- **Precision**

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

Sample	*Mean (µg/ml)*	*Standard Deviation (µg/ml)*	*CV (%)*
1	7.14	0.50	7.0
2	21.17	1.35	6.4

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

Sample	*Mean (µg/ml)*	*Standard Deviation (µg/ml)*	*CV (%)*
1	5.27	0.43	8.2
2	17.78	1.29	7.3

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- **Spiking Recovery**

Serum samples were spiked with different amounts of human Adiponectin and assayed.

Sample	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	4.36	-	-
	8.48	9.36	91
	12.25	14.36	85
	23.12	24.36	95
2	6.90	-	-
	12.29	11.90	103
	15.23	16.90	90
	25.83	26.90	96

- **Dilution Linearity**

Serum samples (30 times diluted) were further serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	-	14.79	-	-
	1:2	7.87	9.63	90
	1:4	3.56	4.82	89
	1:8	1.84	2.41	94
2	-	23.39	-	-
	1:2	11.50	11.70	98
	1:4	5.69	5.85	97
	1:8	2.95	2.92	101

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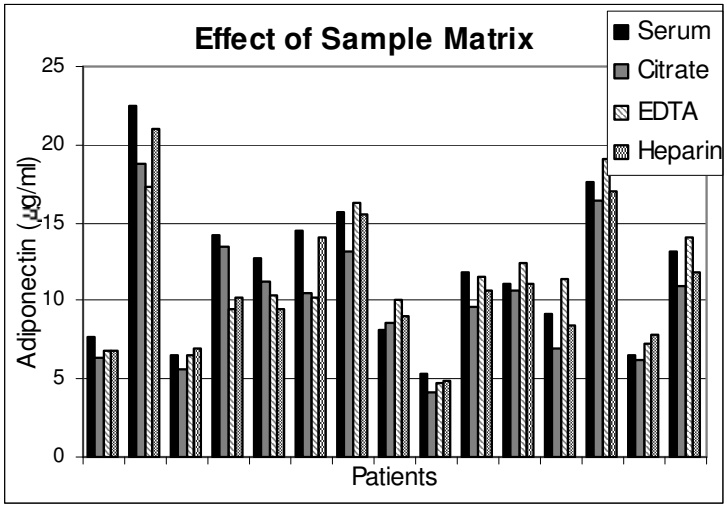
- **Serum/ Plasma Samples**

Citrate, EDTA and heparin plasmas were compared to respective serum samples obtained from healthy persons (n = 15) in the same time.

Sample (n = 15)	*Mean Adiponectin (µg/ml)*	*Plasma/Serum (%)*
Serum	11.77	-
Citrate Plasma	10.17	86.3
EDTA Plasma	11.15	94.7
Heparin Plasma	10.99	93.3



- **Normal Values**

The following results were obtained when serum samples from 335 healthy persons were analysed with BioVendor's Human Adiponectin ELISA. However, every laboratory should establish the own values.

Gender	*BMI (kg/m²)*	*n*	*Mean (µg/ml)*	*SD (µg/ml)*
Men	< 25	41	10.9	4.0
	25-30	52	8.8	4.0
	> 30	23	8.3	2.8
	total	115	9.5	3.9
Women	< 25	92	13.6	5.4
	25-30	56	13.9	8.6
	> 30	57	11.4	3.8
	total	220	13.2	6.1

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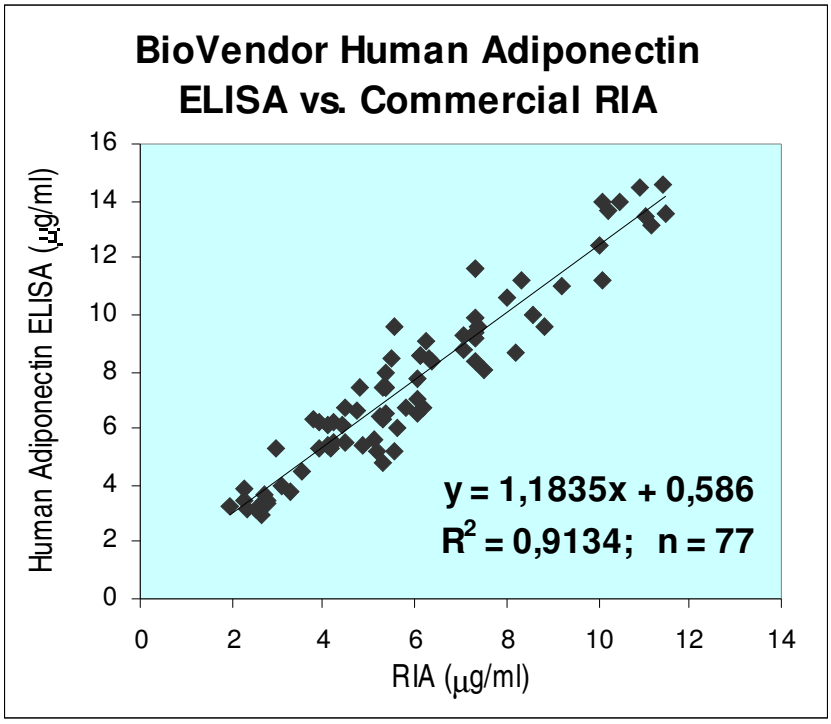
- **Specificity**

The assay recognizes natural and recombinant human Adiponectin (full-length protein, mutation-modified trimer-only-forming protein, and globular domain).
Adiponectin was measured in some of adipose tissue extracts, however most of the extract adiponectin levels were bellow the assay detection limit.
No *cross-reactivity* has been observed for human Leptin, Leptin Receptor and Resistin at 100 ng/ml.
No *interfererence* has been observed for Hemoglobin (5 mg/ml), Bilirubin-mixed isomers (0.4 mg/ml) and Triglycerides (0.25 mg/ml).
Among *animal species*, specific signal was observed (it exhibited dilution linearity) in Rhesus monkey and Cynomolgus monkey sera. The signal was equivalent to 15-19 µg/ml of human adiponectin.
No signal has been obtained when sera of the following *species* were measured in the assay: dog, sheep, goat, horse, cow, pig, rabbit, hamster, mouse, and rat.

- **Method Comparison**

The BioVendor's Human Adiponectin ELISA was compared to other commercial immunoassays, measuring of 77 or 68 serum samples, in radioimmunoassay (RIA) or ELISA, respectively, gave the following correlation graphs.



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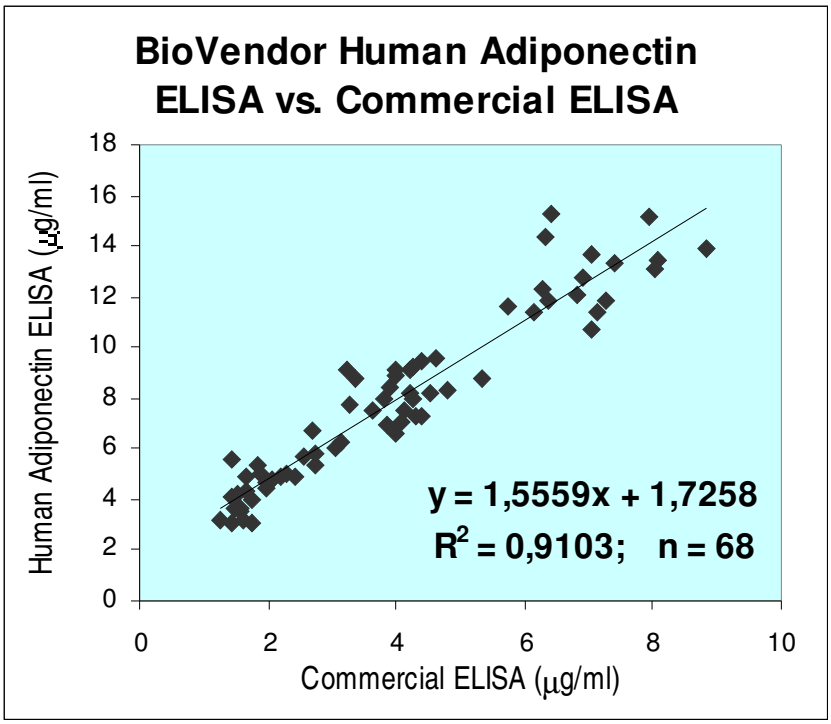
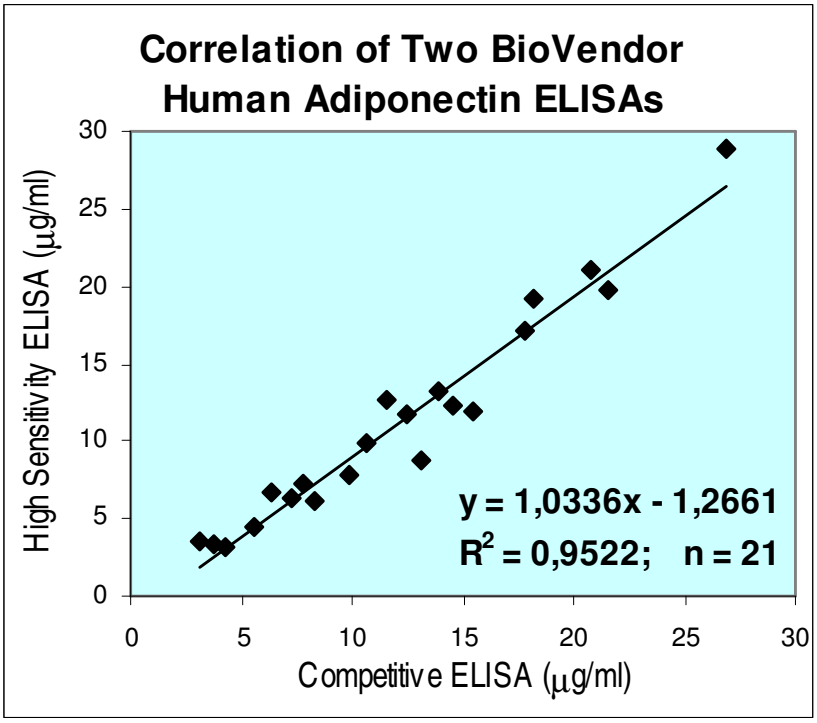


The BioVendor's Human Adiponectin ELISA, High Sensitivity (a sandwich ELISA, RD191023100R) was compared with the other BioVendor's Human Adiponectin ELISA (a competitive ELISA, RD195023100R), measuring 21 serum samples. The following correlation graph was obtained.



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14. Troubleshooting and FAQs

1/ Weak signal in all wells

· Ommiting a reagent or a step
· Improper preparation or storage of a reagent
· Assay performed before reagents were allowed to reach room temperature

2/ High signal and background in all wells

Possible explanations:
· Improper or inadequate washing
· Overdeveloping; incubation time should be reduced before addition of Stop Solution

3/ High coefficient of variation (CV)

Possible explanation:
· Improper or inadequate washing

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

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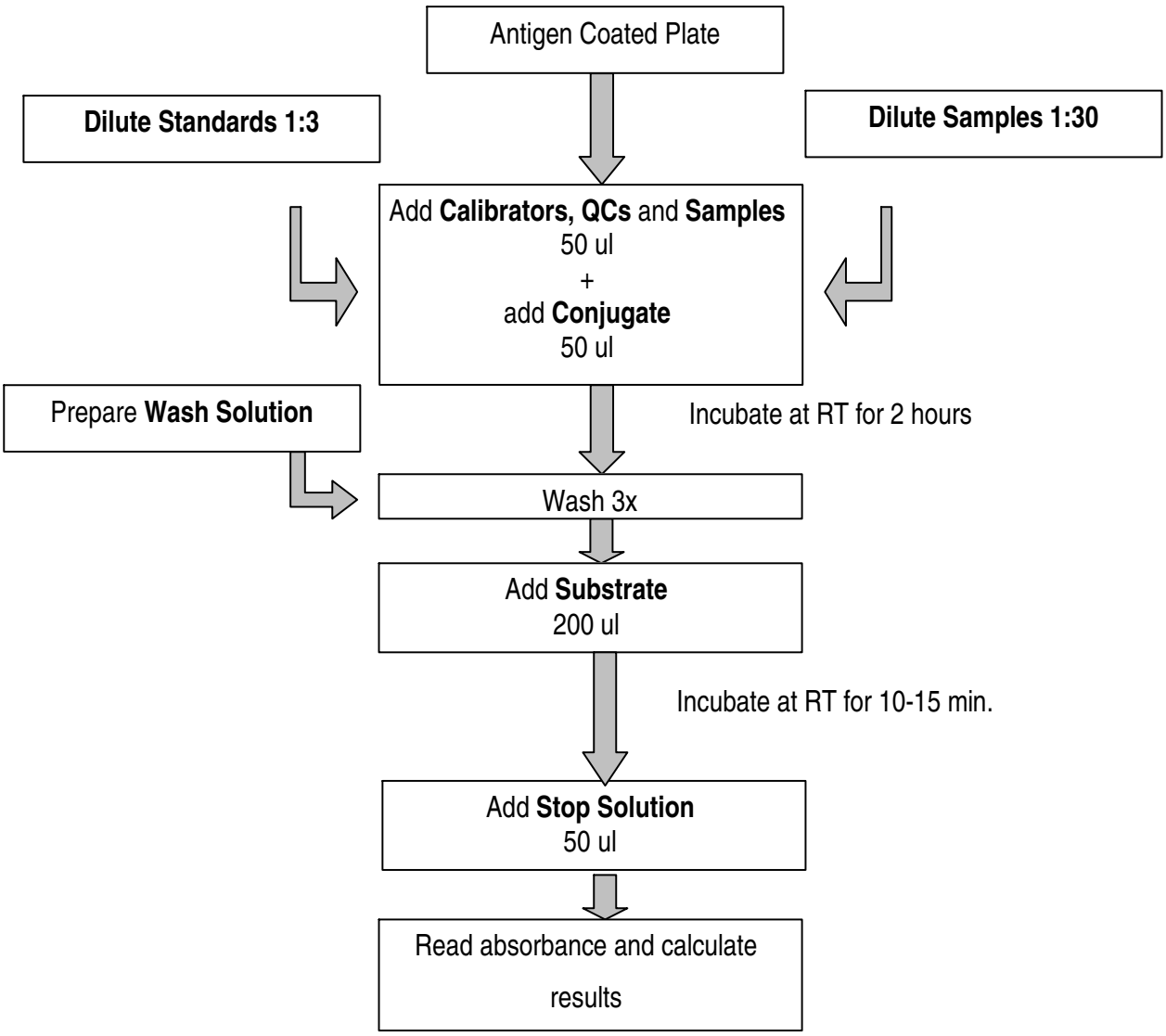
Assay Procedure Summary



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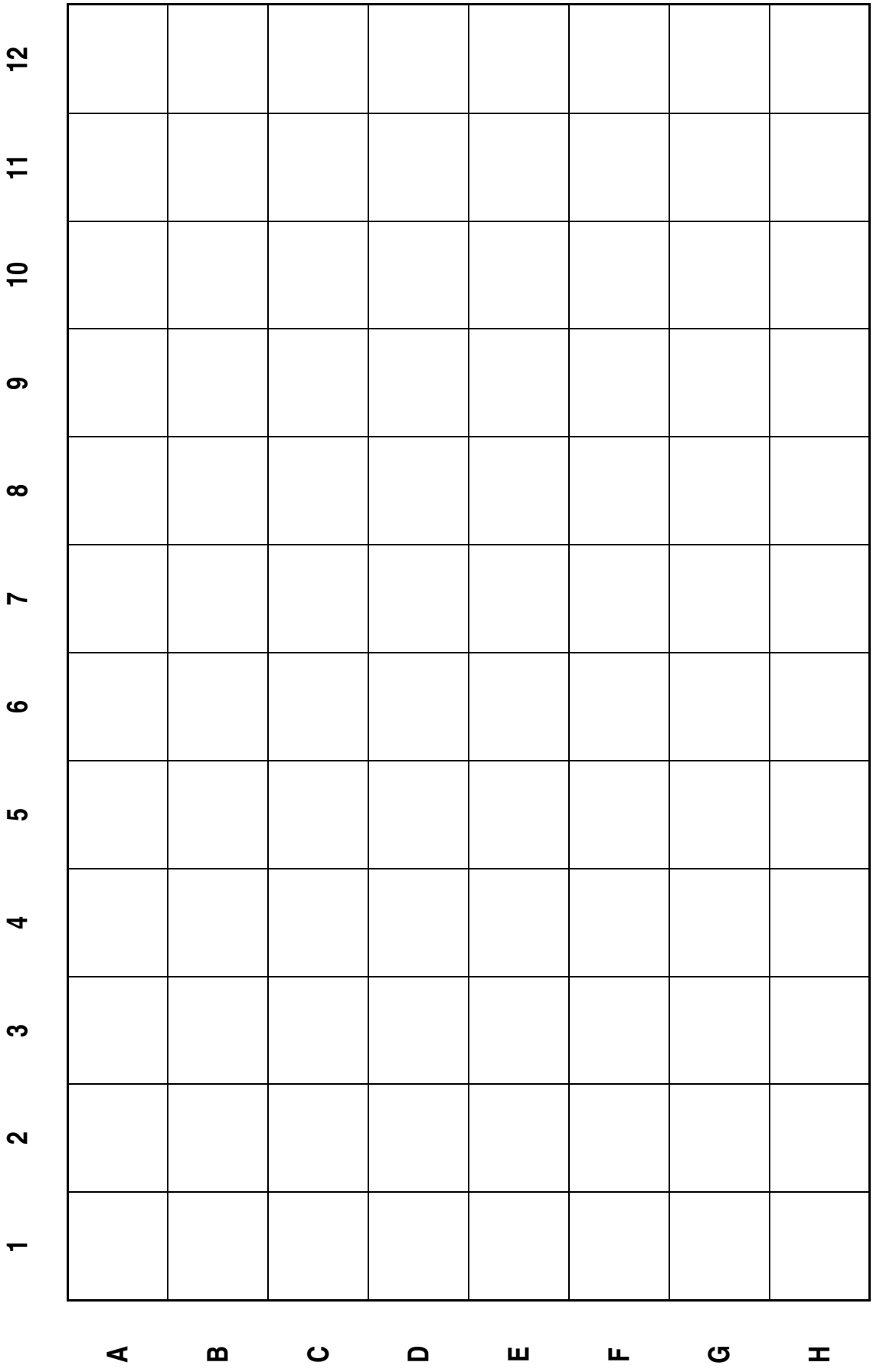

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Notes: