



Leptin (human) ELISA Kit

Manufactured by BioVendor.

ALX-850-044-KI01

96 wells (~80 tests)

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For laboratory use only. Not for human or diagnostic use.

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

1. Intended Use

The Human Leptin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human leptin in serum, plasma and tissue culture medium. It is intended for *in vitro* diagnostic use.

Features

- The total assay time is less than four hours.
- The kit measures total serum leptin.
- 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 and Quality Controls).

2. Storage, Expiration

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

3. Summary

Leptin, the product of the *ob* (obese) gene, is a single-chain 16 kDa protein consisting of 146 amino acid residues. Leptin is produced mainly in the adipose tissue, and is considered to play an important role in appetite control, fat metabolism and body weight regulation. It targets the central nervous system, particularly hypothalamus, affecting food intake. The primary effect of leptin appears to be mediated by leptin receptors expressed mainly in the hypothalamus. In humans, leptin levels correlate with body mass index (BMI) and percentage body fat, and are elevated even in obese individuals. Leptin has a dual action; it decreases the appetite and increases energy consumption, causing more fat to be burned. Leptin is secreted in circadian fashion with nocturnal rise in both lean and obese patients.

Mutations of the *ob* gene resulting in leptin deficiency are the cause of obesity in the *ob/ob* mice. Endogenous leptin can normalize their body weight. In contrast, high levels of leptin in obese human subjects point to an insensitivity to endogenous leptin.

Other factors in addition to the amount of body fat appear to regulate leptin action: insulin, glucocorticoids, catecholamines and sex hormones. Studies have shown that leptin may be linked to reproductive function.

4. Test Principle

In the BioVendor's Human Leptin ELISA, standards, quality controls and samples of sera are incubated in microtitration wells coated with anti-human leptin antibody. After a thorough wash, anti-human leptin antibody labelled with horseradish peroxidase (HRP) is added to the wells and incubated with the immobilized antibody-leptin complex. Following another washing step, the remaining HRP-conjugated antibody is allowed to react with the substrate tetramethylbenzidine. 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 leptin. A standard curve is constructed by plotting absorbance values versus leptin concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5. Precautions

- For *in vitro* diagnostic use.
- 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 tests can guarantee the complete absence of infectious agents.
- Avoid contact with the acidic Stop Solution and Substrate (TMB) Solution, which contains hydrogen peroxide. In case of contact with the Stop Solution and the Substrate Solution wash skin thoroughly with water and seek medical attention, when necessary.
- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- The materials must not be pipetted by mouth.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- Do not mix different lot numbers of any kit components.

6. Reagents Supplied

<i>Cat. No.</i>	<i>Kit Components</i>	<i>Quantity</i>
C011111	Antibody Coated Microtiter Strips (coated with polyclonal Anti-Leptin Ab), sealed	96 wells
C012111	Conjugate Solution (polyclonal Anti-Leptin Antibody, Horseradish Peroxidase Conjugate), ready to use	13 ml
C013111	Human Leptin Standards (1, 2, 5, 10, 20, 50 ng/ml)	0.35 ml /vial
C014151	Quality Control High, lyophilized	1 vial
C014251	Quality Control Low, lyophilized	1 vial
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
-	Instruction Manual + Certificate of Analysis	1 pc

7. Materials Required but Not Supplied

- Test tubes for diluting samples
- Precision pipettes to deliver 50-200 µl
- Multichannel pipette 100 µl
- Microplate reader with 450 ± 10 nm filter
- Orbital microplate shaker capable of approximately 300 rpm
- Software package facilitating data generation and analysis
- Orbital microplate shaker capable of approximately 300 rpm
- Microtitration plate washer (optional) [Manual washing is possible but not preferable.]
- 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 Quality Controls (lyophilized) 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.

Quality Controls:

Reconstitute each vial of Quality Control with 0.35 ml of distilled water at least 30 minutes prior to use. Storage conditions for aliquots are – 20°C.

Dilute the reconstituted Quality Controls 1:3 with Dilution Buffer prior to use (e.g. 100 µl Quality Control + 200 µl Dilution Buffer for duplicates).

Do not store the diluted Quality Controls.

Human Leptin Standards:

Dilute Standards 1:3 with Dilution Buffer prior to use (e.g. 100 µl standards + 200 µl Dilution Buffer for duplicates). Do not store the diluted standard solutions.

Wash Solution:

Dilute 100 ml of Wash Solution Concentrate with 900 ml deionized (distilled) water.

Stability and storage:

The diluted Wash Solution is stable for 1 month when stored at 2-8°C.

9. Preparation of Samples

Dilute samples 1:3 with Dilution Buffer (e.g. 50 µl sample + 100 µl Dilution Buffer when assaying samples in singlets, or preferably 100 µl sample + 200 µl 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 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).
- 4) Add 100 μ l of Conjugate Solution.
- 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).
- 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 minutes at room temperature. (The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C). No shaking!
- 9) Stop the colour development by adding 100 μ l of Stop Solution.
- 10) Determine the absorbance by reading the plate at 450 nm. (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 leptin 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 leptin (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).

Standards, Quality Controls and samples are diluted with Dilution Buffer at the same ratio. Therefore, multiplying the respective results by the dilution factor is not necessary.

12. Limits of Assay

Results exceeding 50 ng/ml should be repeated with more diluted sample (e.g. 1:5). Dilution factors need to be taken into consideration in calculating the concentration of leptin.

13. Performance Characteristics

Typical analytical data of BioVendor Human Leptin ELISA, Clinical Range are presented in this chapter.

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

- **Sensitivity**

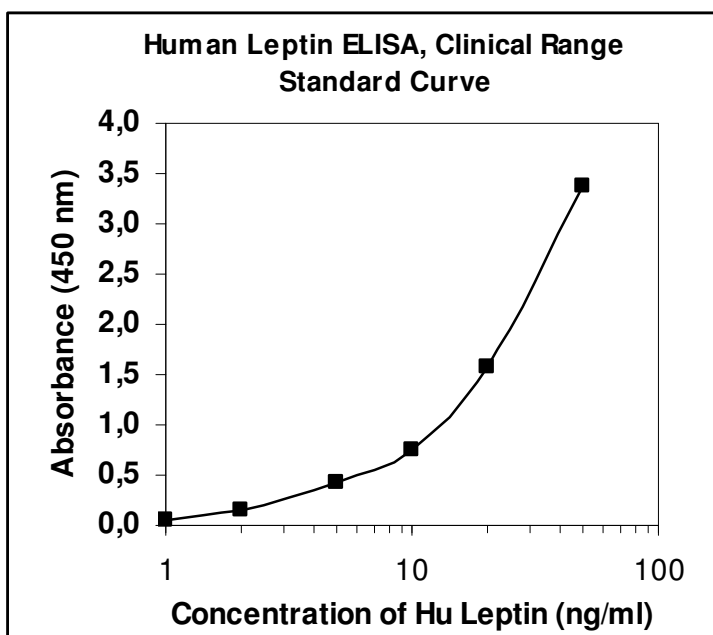
The limit of detection (defined as human leptin concentration giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{\text{blank}} + 3xSD_{\text{blank}}$) is defined as follows:

Analytical Limit of Detection is calculated from the real leptin values in wells and is 0.17ng/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 3 = 0.5 ng/ml

*Dilution Buffer is pipetted into blank wells.



- **Specificity**

The antibodies used in the Human Leptin ELISA kit are highly specific to human Leptin. No signal was found to the following animal sera: rabbit, mouse, pig, rhesus monkey and dog. However, we have found crossreactivity with goat, hamster, bovine, horse, sheep and rat serum. For details please contact us at info@biovendor.com.

- **Precision**

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>Standard Deviation (ng/ml)</i>	<i>CV (%)</i>
1	3.54	0.27	7.5
2	13.63	0.41	3.0
3	25.44	1.38	5.4
4	25.58	1.68	6.7

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>Standard Deviation (ng/ml)</i>	<i>CV (%)</i>
1	5.41	0.50	9.2
2	8.65	0.68	7.8
3	13.9	0.95	6.8
4	25.12	0.81	3.2

- **Spiking Recovery**

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

<i>Sample</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	7.89	-	-
	10.14	11.69	86.7
	15.12	16.47	91.8
	22.17	24.14	91.8
2	12.95	-	-
	18.26	16.75	109.0
	19.55	21.53	90.8
	27.20	29.20	93.2
3	8.09	-	-
	10.92	12.53	87.2
	14.31	16.43	87.1
	20.37	23.03	88.4
4	13.84	-	-
	16.38	18.28	89.6
	18.08	22.18	81.5
	26.71	28.78	92.8

- **Linearity**

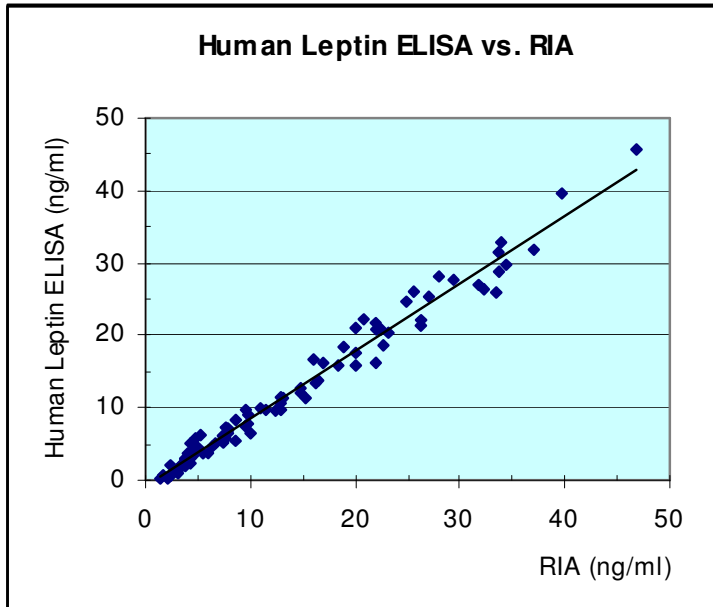
Serum samples were diluted with Dilution Buffer and assayed.

<i>Sample</i>	<i>Dilution</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	-	13.27	-	-
	1:2	7.62	6.64	114.8
	1:4	3.76	3.32	113.3
	1:8	1.58	1.66	95.3
2	-	15.49	-	-
	1:2	8.39	7.75	108.3
	1:4	3.93	3.87	101.5
	1:8	2.31	1.94	119.3
3	-	15.23	-	-
	1:2	7.78	7.62	102.2
	1:4	3.68	3.81	96.7
	1:8	1.84	1.90	96.7
4	-	27.76	-	-
	1:2	13.69	13.88	98.6
	1:4	6.87	6.94	99.0
	1:8	4.10	3.47	118.2

- **Method Comparison**

The BioVendor's Human Leptin ELISA was compared to a commercial RIA. Linear regression analysis of the results yielded the following results.

$$\text{ELISA} = 0.94 \times \text{RIA} - 0.9 \quad r = 0.97$$



14. Definition of Leptin Calibrator

A recombinant protein is used as the calibrator. The recombinant human leptin is a 16 kDa protein containing 147 amino acid residues.

15. 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)

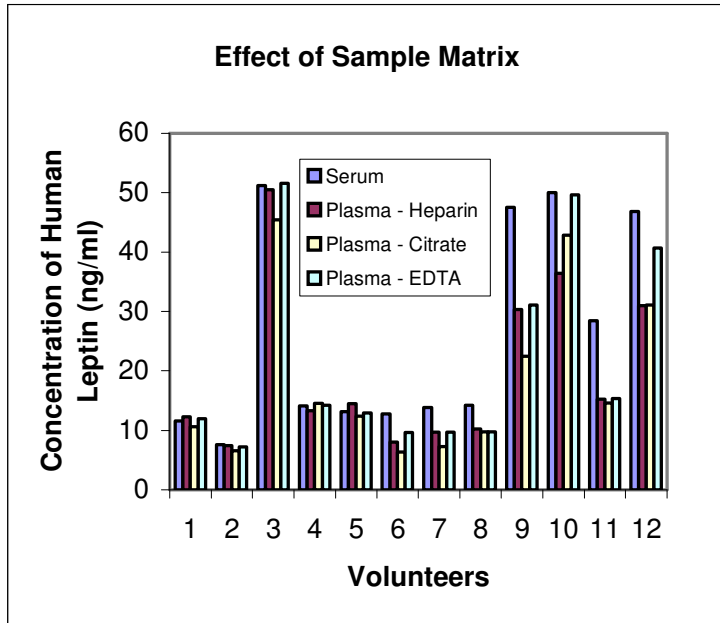
Possible explanation:

- Improper or inadequate washing

4/ Effect of sample matrix (serum/plasma)

Samples from 12 patients were taken and treated by different methods, results shown below:

Volunteer No.	Serum (ng/ml)	Plasma (ng/ml)		
		Heparin	Citrate	EDTA
1	11.56	12.24	10.63	12.01
2	7.59	7.39	6.61	7.23
3	51.18	50.50	45.46	51.58
4	14.13	13.27	14.52	14.21
5	13.16	14.47	12.42	12.96
6	12.73	8.02	6.35	9.63
7	13.86	9.73	7.27	9.68
8	14.22	10.22	9.75	9.79
9	47.52	30.32	22.45	31.12
10	50.00	36.45	42.79	49.6
11	28.51	15.27	14.64	15.4
12	46.81	30.95	31.13	40.7



Mean values of human leptin serum, heparin plasma, citrate plasma and EDTA plasma:

Sample (n=12)	Mean (ng/ml)	Plasma / Serum (%)
Serum	25.9	-
Plasma – Heparin	19.9	76.7
Plasma – Citrate	18.7	72.0
Plasma - EDTA	22.0	84.8

5/ Effect of freezing/thawing on the concentration of Human Leptin in samples

No decline was observed in concentration of human Leptin in serum and plasma samples after repeated (5x) freezing/thawing cycles.

Sample	Number of freezing/thawing cycles	Serum (ng/ml)	Plasma (ng/ml)		
			Heparin	Citrate	EDTA
1	1x	7.4	7.1	6.6	6.4
	3x	10.2	7.2	6.3	8.5
	5x	12.8	7.5	6.3	9.4
2	1x	9.4	14.0	13.6	13.5
	3x	13.3	14.2	12.3	10.7
	5x	13.9	11.9	11.0	11.8

6/ Stability of samples at 4° C

Samples should be stored at -20° C. However, no decline was observed in concentration of leptin in serum and plasma samples when stored at 4° C for 1 week. To avoid microbial contamination, add NaN₃ to a final concentration 0,1% to the samples.

Sample	Incubation: Temperatu re Period	Serum (ng/ ml)	Plasma (ng/ ml)		
			Heparin	Citrate	EDTA
1	-20° C	26.9	33.5	27.4	45.4
	4° C. 7 day	28.0	33.2	27.8	44.3
	4° C. 14 days	26.0	30.1	34.1	40.3
2	-20° C	32.2	41.3	45.4	42.9
	4° C. 7 day	35.5	45.4	41.2	39.8
	4° C. 14 days	33.8	37.9	34.8	32.8

7/ Why are calibrator solutions diluted 1:3 prior to use in ELISA and what does it mean for calculation of results?

The dilution buffer suppresses the matrix effect of samples. It is a common practice to use the same dilution for samples and calibrators because of simple calculation of results: concentrations of samples can be read directly off the curve without considering a dilution factor. Thus the same dilution 1:3 is used for calibrators, controls and serum/plasma samples in Human Leptin ELISA.

Samples exceeding human leptin level of 50 ng/ml should be measured at higher degree of dilution (e.g. 1:9) and dilution factors need to be taken into consideration when calculating human leptin concentrations then (the dilution factor is 3 in this case).

16. References

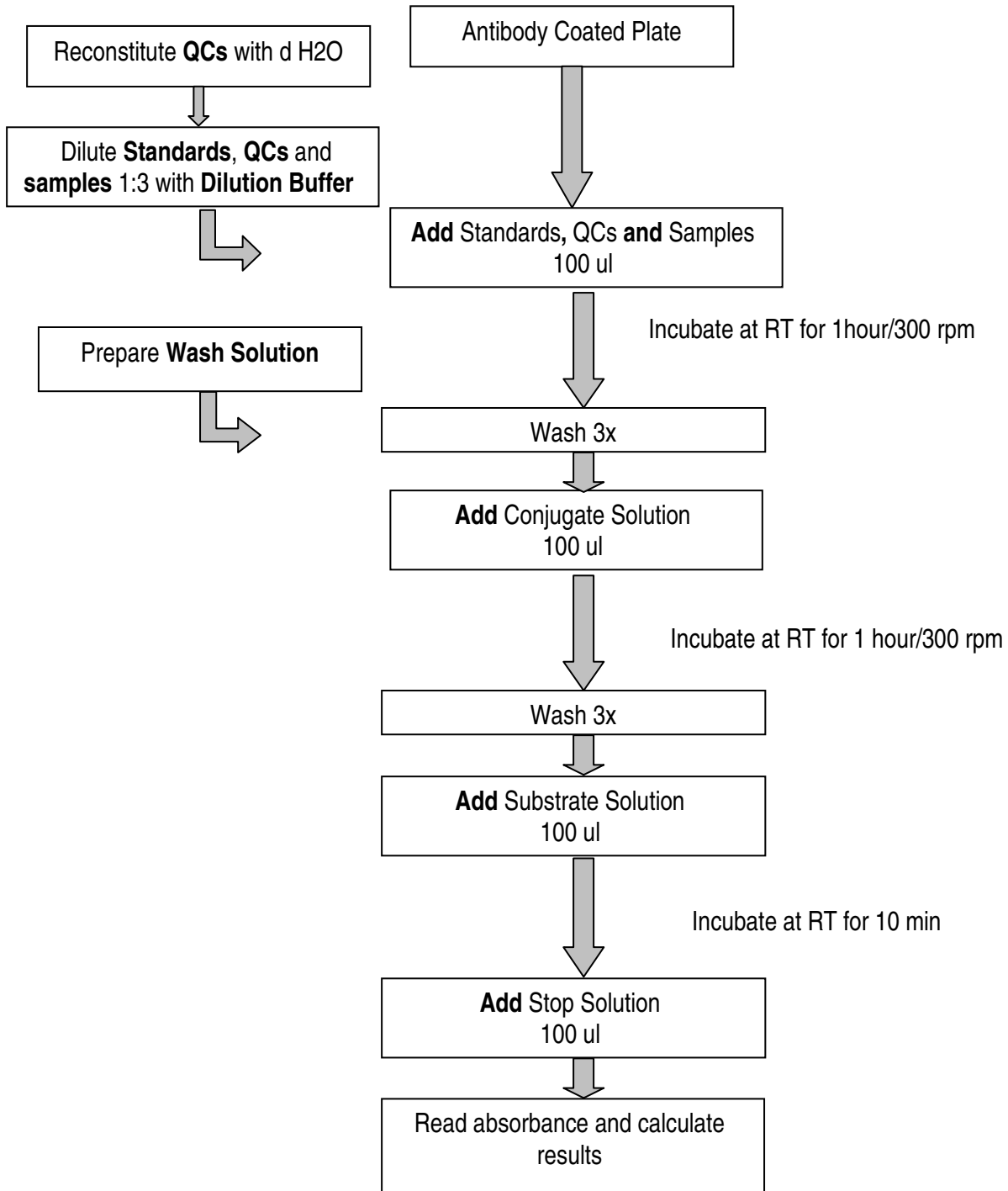
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Assay Procedure Summary



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