



Resistin (human) ELISA Kit

Manufactured by BioVendor.

ALX-850-297-KI01

96 wells (~80 tests)

(Version 3: July 07, 2008)

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

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

1. INTENDED USE

The ALX-850-297 Human Resistin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human resistin.

»» Features

- **It is intended for research use only.**
- The total assay time is less than 4 hours.
- The kit measures total resistin in serum, plasma (heparin, citrate or EDTA), tissue culture medium, synovial fluid and cerebrospinal fluid (CSF).
- Assay format is 96 wells.
- Quality Controls are human serum based. No animal sera are used.
- 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

Resistin, a product of the RSTN gene, is a peptide hormone belonging to the class of cysteine-rich secreted proteins which is termed the RELM family, and is also described as ADSF (Adipose Tissue-Specific Secretory Factor) and FIZZ3 (Found in Inflammatory Zone). Human resistin contains 108 amino acids as a prepeptide, and its hydrophobic signal peptide is cleaved before its secretion. Resistin circulates in human blood as a dimeric protein consisting of two 92 amino acid polypeptides, which are disulfide-linked via Cys26.

Resistin may be an important link between obesity and insulin resistance. Mouse resistin, specifically produced and secreted by adipocyte, acts on skeletal muscle myocytes, hepatocytes and adipocytes themselves so that it reduces their sensitivity to insulin. Steppan et al. have suggested that resistin suppresses the ability of insulin to stimulate glucose uptake. They have also suggested that resistin is present at elevated levels in blood of obese mice, and is down regulated by fasting and antidiabetic drugs. Way et al., on the other hand, have found that resistin expression is severely suppressed in obesity and is stimulated by several antidiabetic drugs.

Other studies have shown that mouse resistin increases during the differentiation of adipocytes, but it also seems to inhibit adipogenesis. In contrast, the human adipogenic differentiation is likely to be associated with a down regulation of resistin gene expression. Recent studies have shown that human resistin is expressed also in macrophages and may be a novel link between inflammation and insulin resistance.

Areas of investigation:

Energy metabolism and body weight regulation

4. TEST PRINCIPLE

In the Human Resistin ELISA, Standards, Quality Controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human resistin antibody. After 60 minutes incubation and washing, biotin-labelled second polyclonal anti-human resistin antibody is added and incubated with captured resistin for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 60 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 spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of resistin. A standard curve is constructed by plotting absorbance values against concentrations of Standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- 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.
- 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

<i>Kit Components</i>	<i>State</i>	<i>Quantity</i>
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labeled Antibody	ready to use	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
Dilution Buffer	ready to use	20 ml
Wash Solution Concentrate (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 50-1000 μ l with disposable tips
- Multichannel pipette to deliver 100 μ l 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 \pm 10 nm filter
- 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 desiccant and seal carefully. Remaining Microtiter Strips are stable 3 month stored at 2-8°C and protected from the moisture.

Biotin Labelled Antibody

Streptavidin-HRP conjugate

Dilution Buffer

Substrate Solution

Stop Solution

Stability and storage:

Opened reagents are stable 3 month when stored at 2-8°C.

- Assay reagents supplied concentrated or lyophilized:

Human Resistin 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 occasionally gently shaking (not to foam). The resulting concentration of the resistin in the stock solution is **50 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	50 ng/ml
500 µl of stock	750 µl	20 ng/ml
500 µl of 20 ng/ml	500 µl	10 ng/ml
500 µl of 10 ng/ml	500 µl	5 ng/ml
500 µl of 5 ng/ml	750 µl	2 ng/ml
500 µl of 2 ng/ml	500 µl	1 ng/ml

Dilute each concentration of standard 3x with Dilution Buffer prior to the assay, e.g. 50 µl of standard + 100 µl of Dilution Buffer for singlets, or preferably 100 µl of standard + 200 µl of Dilution Buffer for duplicates. Mix well (not to foam).

Stability and storage:

Standard stock solution (50 - 1 ng/ml) should be aliquoted and frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.

Do not store the diluted Standard solutions (3x).

Quality Controls High, Low

Reconstitute each Quality Control (High and Low) with 350 µl of Dilution Buffer just prior to the assay. Let it dissolve at least 30 minutes with occasional gentle shaking (not to foam).

Dilute Quality Controls prior to the assay 3x with Dilution Buffer, e.g. 50 µl of standard + 100 µl of Dilution Buffer for singlets, or preferably 100 µl of standard + 200 µl of Dilution Buffer for duplicates

Stability and storage:

The reconstituted Quality Controls must be used immediately or aliquoted and frozen at -20°C for 3 month. Avoid repeated freeze/thaw cycles.

Do not store the diluted Quality Controls solutions (3x).

Wash Solution Concentrate (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 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 resistin (homodimeric) in serum or plasma.

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.

Dilute samples 3x with Dilution Buffer just prior to the assay (e.g. $50\ \mu\text{l}$ of sample + $100\ \mu\text{l}$ of Dilution Buffer for singlets, or preferably $100\ \mu\text{l}$ of sample + $200\ \mu\text{l}$ of Dilution Buffer for duplicates). **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.

Do not store the diluted samples (3x).

See Chapter 13 for stability of serum or plasma samples if stored at $2-8^{\circ}\text{C}$ and effect of freezing/thawing on the concentration of human resistin.

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

Ask for protocol at if assaying tissue culture medium, synovial fluid and cerebrospinal fluid (CSF).

11. ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards, Quality Controls, Dilution Buffer (=Blank) 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). After final wash, invert and tap the plate strongly against paper towel.
4. Add **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 **3-times** with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells **3-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 the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance by reading the plate at 450 nm. The absorbance should be read within 5 minutes following step 12.

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 resistin 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	Standard 50	QC Low	Sample 8	Sample 16	Sample 24	Sample 32
B	Standard 20	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Standard 10	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Standard 5	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Standard 2	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Standard 1	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	QC High	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 at 450 nm (Y) of Standards against log of the known concentration (X) of Standards, using the four-parameter algorithm. Results are reported as concentration of resistin ng/ml in samples.

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

Samples, Quality Controls and Standards are all diluted 3x prior to analysis, so there is no need to take this dilution factor into account.

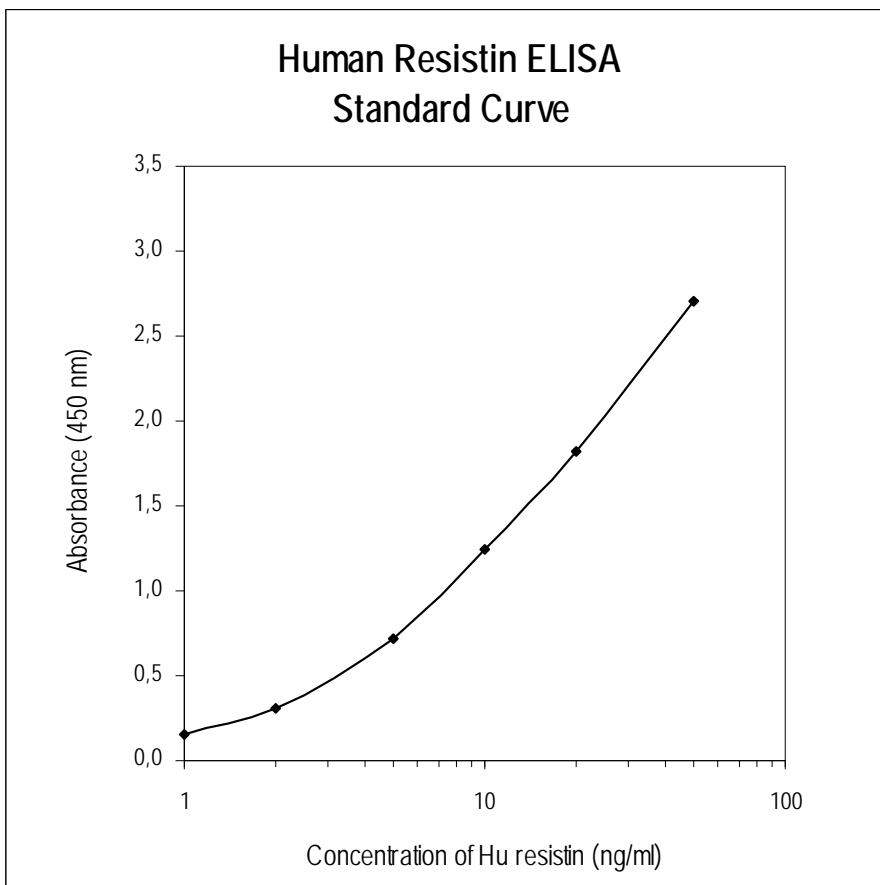


Figure 2: Typical Standard Curve for Human Resistin ELISA.

13. PERFORMANCE CHARACTERISTICS

➤➤ Typical analytical data of Human Resistin 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: $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$) is calculated from the real human resistin values in wells and is 0.033 ng/ml.

*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding resistin level of 50 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the resistin concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human resistin with no detectable crossreactivities to human leptin, leptin receptor, adiponectin, TNF-alfa, RELM-beta, A-FABP and E-FABP at 100 ng/ml and IL-6, AGRP and Asp (C3adesArg) at 2 ng/ml.

Sera of several mammalian species were measured in the assay. See results below.

<i>Mammalian serum sample</i>	<i>Observed crossreactivity</i>
Bovine	no
Goat	no
Hamster	no
Rat	no
Horse	yes
Monkey	yes
Mouse	no
Pig	yes
Rabbit	no
Sheep	no
Cat	no
Dog	no

- Precision**

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
1	7.53	0.21	2.8
2	11.35	0.39	3.4

Inter assay (Run-to-Run) (n=3)

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
1	6.46	0.33	5.1
2	13.35	0.93	6.9

- Spiking Recovery**

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

<i>Sample</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1	5.55	-	-
	8.99	10.55	85.2
	13.35	15.55	85.9
	25.34	25.55	99.2
2	7.47	-	-
	10.88	12.47	87.2
	16.79	17.47	96.1
	26.07	27.47	94.9

- Linearity**

Serum samples were 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	-	12.56	-	-
	2x	6.51	6.28	103.7
	4x	2.98	3.14	94.9
	8x	1.74	1.57	110.8
2	-	28.46	-	-
	2x	14.02	14.23	98.5
	4x	7.27	7.12	102.2
	8x	3.88	3.56	109.1

- **Effect of sample matrix**

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

Results are shown below:

Volunteer No.	Serum (ng/ml)	Plasma (ng/ml)		
		Heparin	Citrate	EDTA
1	14.1	11.2	12.0	13.3
2	7.9	6.6	6.7	6.5
3	11.3	10.2	9.9	12.2
4	9.0	8.3	8.0	9.0
5	5.9	6.2	7.0	8.2
6	9.3	8.9	7.8	9.3
7	6.2	6.3	6.6	7.7
8	5.6	5.9	5.9	5.6
9	5.1	6.0	4.8	5.4
10	6.9	6.5	8.1	7.9
Mean (ng/ml)	8.13	7.61	7.68	8.51
Mean Plasma/Serum (%)	-	99.1	90.2	104.7
Correlation. coeff. R²	-	0.95	0.89	0.86

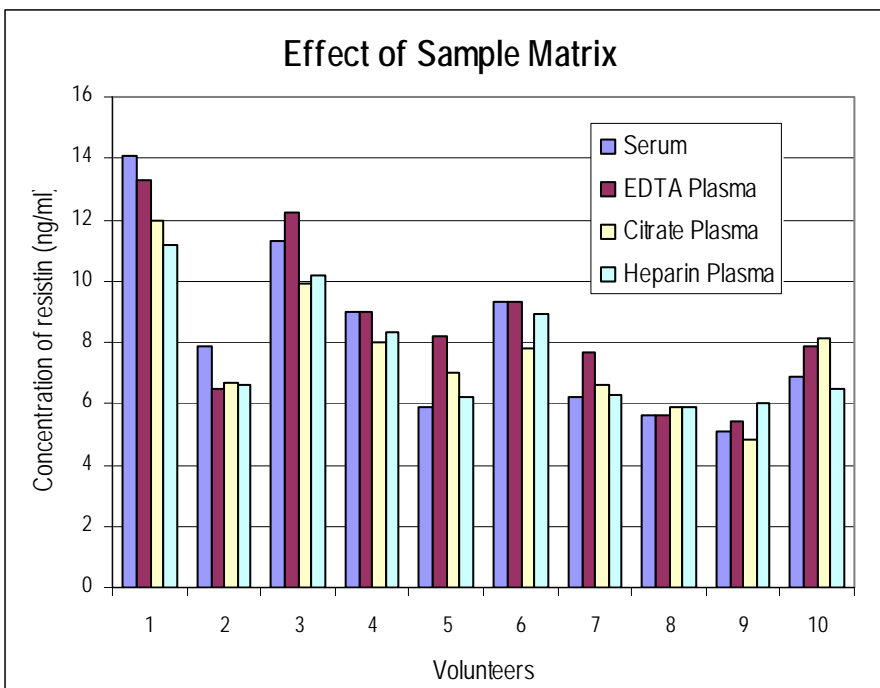


Figure 3: Resistin levels measured using Human Resistin ELISA from 10 individuals using serum, heparin, citrate and EDTA plasma, respectively.

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

Samples should be stored at -20°C. However, no decline in concentration of resistin was observed in serum and plasma samples after 10 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 (ng/ml)	Plasma (ng/ml)		
			Heparin	Citrate	EDTA
1	-20°C	14.1	11.2	12.0	13.3
	2-8°C, 1 day	14.5	13.2	13.1	10.7
	2-8°C, 10 days	14.2	10.9	13.2	12.9
2	-20°C	9.3	8.9	7.8	9.3
	2-8°C, 1 day	10.2	8.4	8.2	9.9
	2-8°C, 10 days	8.8	8.7	7.4	8.7
3	-20°C	5.1	6.0	4.8	5.4
	2-8°C, 1 day	5.1	5.4	4.6	5.7
	2-8°C, 10 days	5.4	5.5	4.1	5.5

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human resistin in serum and plasma samples after repeated (3x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum (ng/ml)	Plasma (ng/ml)		
			Heparin	Citrate	EDTA
1	1x	14.1	11.2	12.0	13.3
	3x	12.8	12.2	11.8	13.1
	5x	11.6	10.5	10.4	11.5
2	1x	9.3	8.9	7.8	9.3
	3x	9.4	8.3	7.3	9.1
	5x	9.2	8.7	7.1	8.4
3	1x	5.1	6.0	4.8	5.4
	3x	5.3	4.9	4.7	4.7
	5x	3.8	4.5	3.9	4.1

14. DEFINITION OF THE STANDARD

A recombinant protein is used as the standard. The recombinant resistin is a 19,5 kDa dimeric protein consisting of two 92 amino acid polypeptide chains which are disulfide-linked.

15. 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

16. REFERENCES

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»» References to this product:







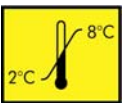

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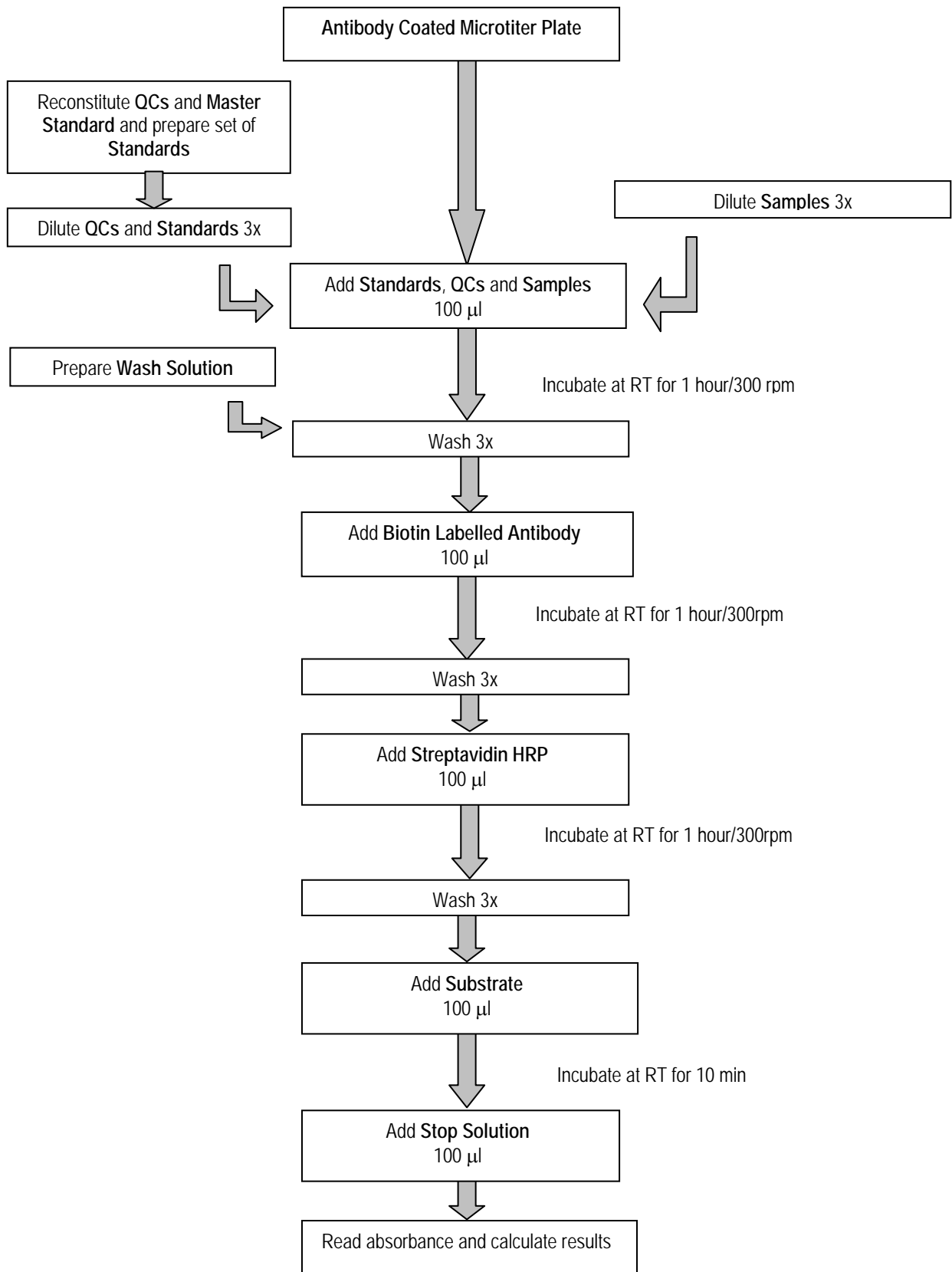
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17. EXPLANATION OF SYMBOLS

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

Assay Procedure Summary



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NOTES

