



Leptin (mouse/rat) ELISA Kit

Manufactured by BioVendor.

ALX-850-317-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 current version of Product Data Sheet enclosed with the kit!

1. INTENDED USE

The Alexis Mouse and Rat Leptin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of mouse and/or rat leptin.

»» Features

- It is intended for research use only.
- The total assay time is less than 3.5 hours.
- The kit measures leptin in serum, plasma and tissue culture media.
- Assay format is 96 wells.
- Quality Controls are mouse and rat serum based. No human sera are used.
- Standards are 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

Leptin is a protein hormone with important effects in metabolism and regulating body weight. It is a single-chain 16 kDa protein consisting of 146 amino acid residues and encoded by the obese (*ob*) gene.

Leptin is expressed predominantly by adipocytes, small amounts of leptin are also secreted by cells in the epithelium of stomach and in the placenta. Leptin's effect on body weight is mediated through effects on hypothalamic centers, where leptin receptors are highly expressed. Leptin has a dual action, it decreases the appetite and increases energy consumption.

A mutations in the *ob* gene of leptin or in the gene of leptin receptor causes hyperphagia, reduced energy expenditure, and severe obesity in the *ob/ob* mice.

Ob gene knockout mice are also characterized by several metabolic abnormalities including hyperglucocorticoidemia, hyperglycaemia, hyperinsulinemia and insulin resistance.

When *ob/ob* mice are treated with injections of leptin, they lose their excess fat and return to normal body weight.

Studies have shown that leptin appears to be a significant regulator of reproductive function. In addition, leptin is involved in bone metabolism and plays a significant role as an immunomodulator.

Areas of investigation:

Energy metabolism and body weight regulation

4. TEST PRINCIPLE

In the Alexis Mouse and Rat Leptin ELISA, Standards, Quality Controls and samples are incubated in microplate wells pre-coated with anti-mouse leptin antibody. After 60 minutes incubation and washing, biotin-labelled polyclonal anti-mouse leptin antibody is added to the wells and incubated with immobilized antibody-leptin complex 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 spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of leptin. 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 animal origin. However, 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

<i>Kit Components</i>	<i>State</i>	<i>Quantity</i>
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody (10x)	concentrated	1.3 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard MOUSE	lyophilized	1 vial
Master Standard RAT	lyophilized	1 vial
Quality Control MOUSE	lyophilized	2 vials
Quality Control RAT	lyophilized	2 vials
Dilution Buffer	ready to use	2x 13 ml
Biotin-Ab Diluent	ready to use	13 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 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
- 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.
 - Use Mouse Leptin Standard to quantify leptin concentration in mouse samples.
 - Use Rat Leptin Standard to quantify leptin concentration in rat samples.
- 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 months stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate

Dilution Buffer

Biotin-Ab Diluent

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:

MOUSE Leptin Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution! 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 Mouse Leptin in the stock solution is 4 000 pg/ml (= M-Std. 4000 pg/ml).

Prepare all concentrations of Mouse Leptin Standards as described below:

<i>Volume of standard</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
M-Std. 4000 pg/ml	-----	4000 pg/ml
250 µl M-Std. 4000 pg/ml	250 µl	2000 pg/ml
250 µl M-Std. 2000 pg/ml	250 µl	1000 pg/ml
200 µl M-Std. 1000 pg/ml	300 µl	400 pg/ml
250 µl M-Std. 400 pg/ml	250 µl	200 pg/ml
250 µl M-Std. 200 pg/ml	250 µl	100 pg/ml

Prepared Standards are ready to use, do not dilute them.

RAT Leptin Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution! 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 Rat Leptin in the stock solution is 4000 pg/ml (= R-Std. 4000 pg/ml).

Prepare all concentrations of Rat Leptin Standards as described below:

<i>Volume of standard</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
R-Std. 4000 pg/ml	-----	4000 pg/ml
250 µl R-Std. 4000 pg/ml	250 µl	2000 pg/ml
250 µl R-Std. 2000 pg/ml	250 µl	1000 pg/ml
200 µl R-Std. 1000 pg/ml	300 µl	400 pg/ml
250 µl R-Std. 400 pg/ml	250 µl	200 pg/ml
250 µl R-Std. 200 pg/ml	250 µl	100 pg/ml

Prepared Standards are ready to use, do not dilute them.

Stability and storage:

250 µl of each standard is sufficient to run the standard curve in duplicates. Reconstituted standards (4000 pg/ml) can be aliquoted and stored at -20 °C until next use (up to 3 months). Avoid repeating freezing/thawing cycles.

Quality Controls MOUSE / RAT

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Controls concentrations!

Reconstitute appropriate Quality Control (MOUSE or RAT) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage:

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

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.

Biotin-Labelled Antibody (10x)

Dilute the Biotin-Labelled Antibody (10x) with the Biotin-Ab Diluent 10-fold. Prepare only the volume needed. Example: 100 µl of Biotin-Labelled Antibody (10x) + 900 µl of Biotin-Ab Diluent = 1 ml of working Biotin-Labelled Antibody solution, sufficient for 1 strip.

Stability and storage:

The diluted Biotin-Labelled Antibody solution is stable for 1 month when stored at 2-8°C. Opened Biotin-Labelled Antibody (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures leptin in serum, plasma and tissue culture media.

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 just prior to the assay 20x with the Dilution Buffer. Example: 7 µl of sample + 133 µl of Dilution Buffer for singlets, or preferably 14 µl of sample+ 266 µl 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. Avoid repeated freeze/ thaw cycles.

Do not store the diluted samples.

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 Standards, Quality Controls, Dilution Buffer (=Blank) and diluted 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 Solution into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 min.**, 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 1: 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.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against absorbent paper or 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	M-Std. 4000	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	M-Std. 2000	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	M-Std. 1000	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	M-Std. 400	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	M-Std. 200	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	M-Std. 100	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	QC Mouse	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
H	Blank	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40

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 leptin pg/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.

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. 500 pg/ml (from standard curve) x 20 (dilution factor) = 10 000 pg/ml = 10 ng/ml).

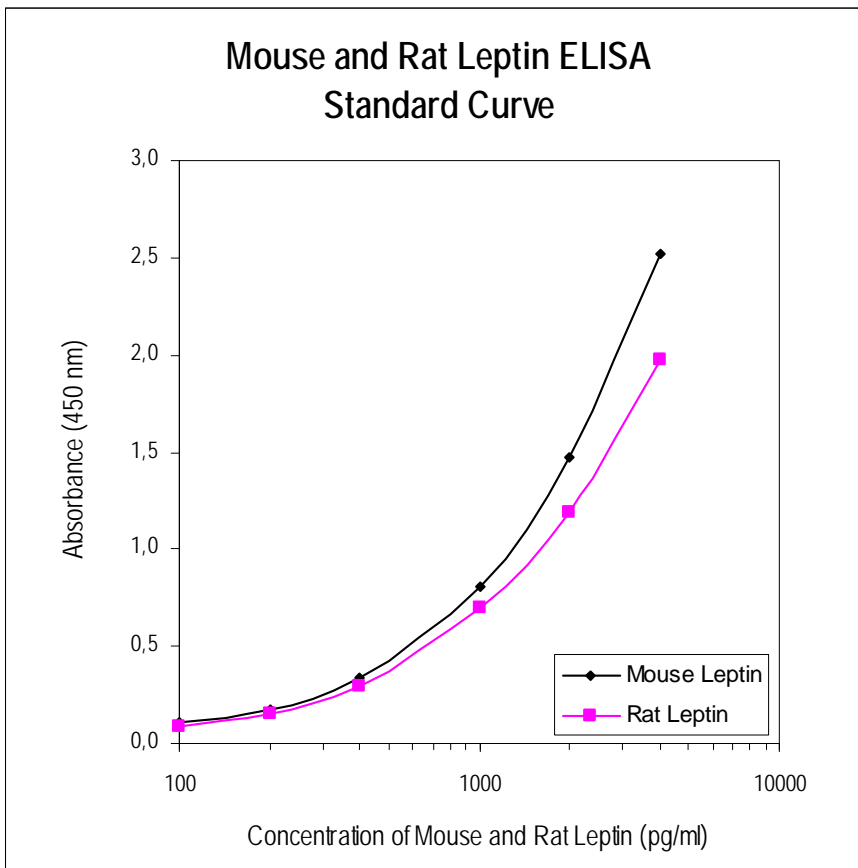


Figure 2: Typical Standard Curve for Mouse and Rat Leptin ELISA.

13. PERFORMANCE CHARACTERISTICS

➤ Typical analytical data of Alexis Mouse/Rat Leptin 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 leptin values in wells and is 30 pg/ml for mouse leptin and 50 pg/ml for rat leptin.

*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding leptin level of 4 000 pg/ml should be repeated with more diluted samples (e.g 40x). Dilution factor needs to be taken into consideration in calculating the leptin concentration.

- **Specificity**

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

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

- **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 mouse	12.31	0.25	2.0
2 mouse	31.48	0.91	2.9
3 rat	9.74	0.18	1.8
4 rat	39.96	0.75	1.9

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
1 mouse	21.32	0.48	2.3
2 rat	17.13	0.76	4.4

- Spiking Recovery**

Serum samples were spiked with different amounts of mouse or rat leptin and assayed.

<i>Sample</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
1 mouse	12.02	-	-
	15.19	16.02	94.8
	18.46	20.02	92.2
	25.90	28.02	92.4
2 mouse	19.56	-	-
	21.86	23.56	92.8
	24.04	27.56	87.2
	32.03	35.56	90.8
3 rat	9.32	-	-
	13.33	13.32	100.1
	16.29	17.32	94.1
	25.47	25.32	100.6
4 rat	19.61	-	-
	22.41	23.61	94.9
	25.25	27.61	95.1
	34.83	35.61	97.8

- 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 mouse	-	34.77	-	-
	2x	16.78	17.39	96.5
	4x	8.35	8.69	96.0
	8x	4.06	4.35	93.4
2 mouse	-	23.44	-	-
	2x	11.69	11.72	99.7
	4x	5.85	5.86	99.8
	8x	2.77	2.93	94.6
3 rat	-	29.67	-	-
	2x	14.53	14.83	98.0
	4x	7.11	7.42	95.8
	8x	3.91	3.71	105.5
4 rat	-	40.78	-	-
	2x	19.86	20.39	97.4
	4x	9.84	10.19	96.6
	8x	5.23	5.10	102.7

14. DEFINITION OF THE STANDARD

Mouse and Rat leptin standards are recombinant *E.coli* expressed proteins.

Mouse leptin standard concentration was determined using the international standard: Leptin, mouse rDNA-derived 1st International Standard NIBSC code 97/626, Version 02, dated April 19 2004.

No rat leptin international standard is available.

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

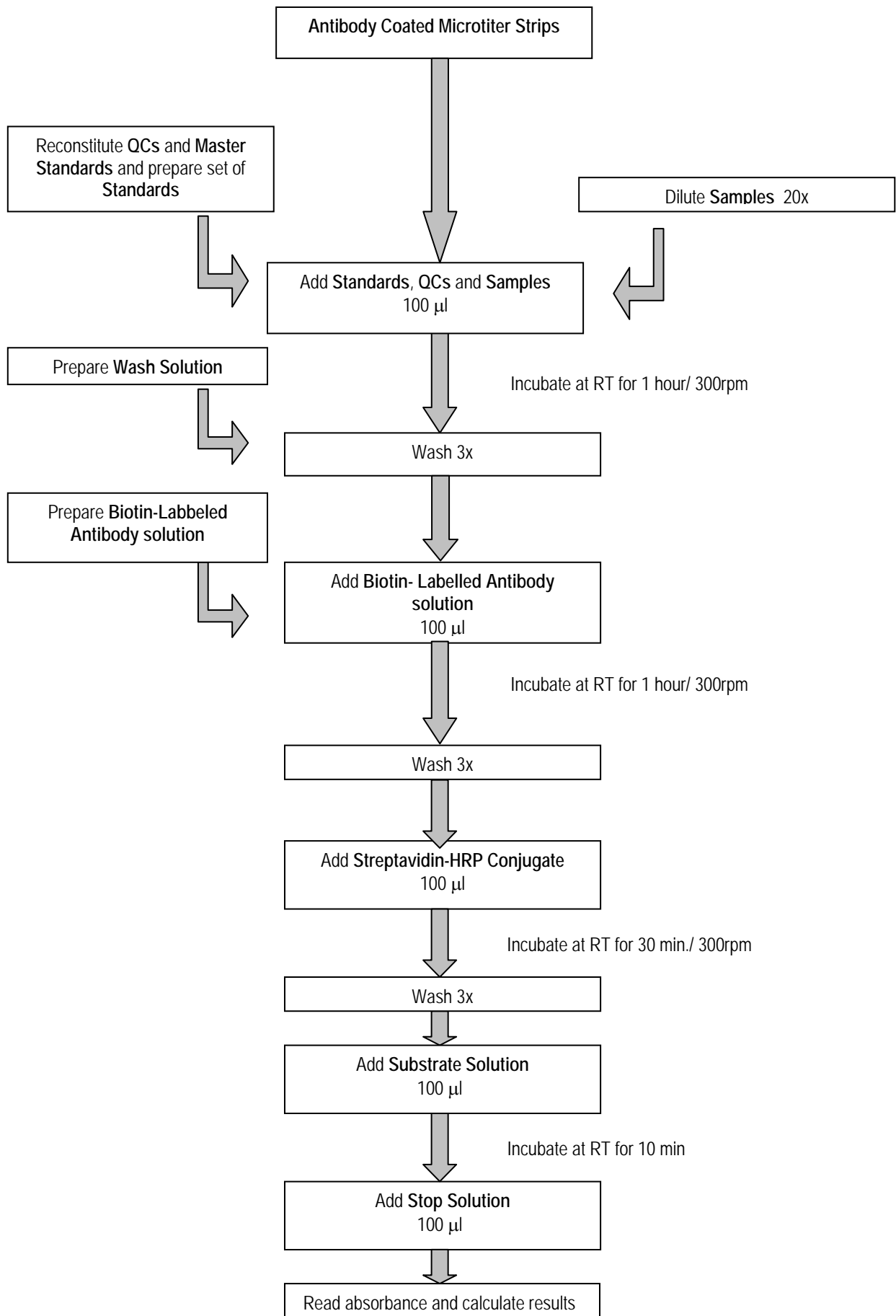
»» References to mouse/rat leptin:

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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



1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
	A	B	C	D	E	F	G	H	

NOTES

