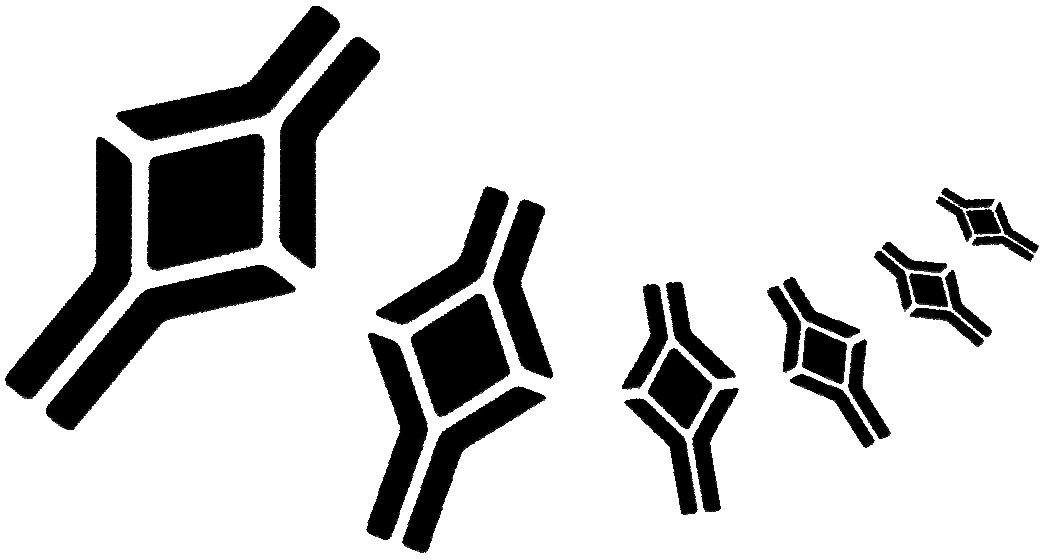
BioVendor
Research
and Diagnostic Products



MOUSE ADIPOCYTE FABP ELISA

Product Data Sheet

Cat. No.: RD291036200R

For Research Use Only

VERSION 03 200411 19

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 RD291036200R Mouse Adipocyte FABP ELISA is a sandwich enzyme immunoassay for the quantitative measurement of mouse AFABP.

Features

- **It is intended for research use only**
- The total assay time is less than 4 hours
- The kit measures total AFABP in mouse serum
- Assay format is 96 wells
- Standard is native serum 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

Protein definition:
Protein name: **Adipocyte FABP**
Synonyms: aP2
AFABP
Fatty acid-binding protein, adipocyte (A-FABP)
Adipocyte lipid-binding protein (ALBP)
A-FABP
P2 adipocyte protein
Myelin P2 protein homolog
3T3-L1 lipid binding protein
422 protein
P15
Gene name:
FABP5
Ap2
Swissprot: P15090
NCBI / Protein: P15090

Adipocyte fatty acid binding protein AFABP is a 15 kDa member of the intracellular fatty acid binding protein (FABP) family, which is known for the ability to bind fatty acids and related compounds (bile acids or retinoids) in an internal cavity. AFABP is expressed in a differentiation-dependent fashion in adipocytes and is a critical gene in the regulation of the biological function of these cells. In mice, targeted mutations in FABP4 (gen also called: aP2 and its protein also called: P2 adipocyte protein, 3T3-L1 lipid binding protein) provide significant protection from hyperinsulinemia and insulin resistance in the context of both dietary and genetic obesity. Adipocytes obtained from AFABP-deficient mice also have reduced efficiency of lipolysis in vitro and in vivo, and these mice exhibited moderately improved systemic dyslipidemia. Recent studies also demonstrated AFABP expression in macrophages upon differentiation and activation. In these cells, AFABP modulates inflammatory responses and cholesterol ester accumulation, and total or macrophage-specific AFABP deficiency confers dramatic protection against atherosclerosis in the apoE-/- mice. These results indicate a central role for AFABP in the development of major components of the metabolic syndrome through its distinct actions in adipocytes and macrophages.
Besides being active within the cell, AFAB appears to be a secreted protein. The extracellular role of secreted AFABP remains to be determined.

Areas of investigation:
Energy metabolism and body weight regulation

4. TEST PRINCIPLE

In the BioVendor Mouse AFABP ELISA, standard and samples are incubated in microplate wells pre-coated with polyclonal anti-mouse AFABP antibody. After two hours incubation and washing, biotin labelled polyclonal anti-mouse AFABP antibody is added and incubated with captured AFABP for one hour. 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. The absorbance is proportional to the concentration of AFABP. 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

- **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 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	lyophilized	1 vial
Biotin-Ab Diluent	ready to use	13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	1 vial
Dilution Buffer	ready to use	2 x 20 ml
Wash Solution Conc. (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 10-1000 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 when stored at 2-8°C and protected from the moisture.

Biotin-Ab Diluent
Streptavidin-HRP Conjugate
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:**

Mouse AFABP 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 the mouse AFABP 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
250 µl of stock	250 µl	25 ng/ml
250 µl of 25 ng/ml	250 µl	12.5 ng/ml
250 µl of 12.5 ng/ml	250 µl	6.25 ng/ml
250 µl of 6.25 ng/ml	250 µl	3.13 ng/ml
250 µl of 3.13 ng/ml	250 µl	1.56 ng/ml
250 µl of 1.56 ng /ml	250 µl	0.78 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
Standard stock solution (50 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.

Biotin Labelled Antibody
Refer to the Certificate of Analysis for current volume of Biotin-Ab Diluent needed for reconstitution of Biotin Labelled Antibody!!!
Reconstitute the lyophilized Biotin Labelled Antibody with Biotin-Ab Diluent. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Add 0.1 ml of reconstituted Biotin Labelled Antibody prior to use in the assay into the remaining amount of Biotin-Ab Diluent and mix gently (not to foam).
Stability and storage:
Reconstituted Biotin Labelled Antibody is stable 3 months when stored at -20°C. Diluted Biotin Labelled Antibody solution is stable 1 month when stored at 4°C.

Wash Solution Conc. (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 mouse AFABP in serum.

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 100x with Dilution Buffer just prior to the assay in two steps as follows:
Dilution A (25x):
Add 10 µl of sample into 240 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.
Dilution B (4x):
Add 100 µl of Dilution A into 300 µl of Dilution Buffer to prepare final dilution (100x). **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

See Chapter 13 for effect of freezing/thawing on the concentration of mouse AFABP.

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, 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. 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 5-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 **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 30 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 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 12.**

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 mouse AFABP 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: 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 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	**Standard 50**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 25**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 12.5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 6.25**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	**Standard 3.13**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	**Standard 1.56**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	**Standard 0.78**	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 (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of mouse AFABP 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.

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. 6.72 ng/ml (from standard curve) x 100 (dilution factor) = 672 ng/ml.

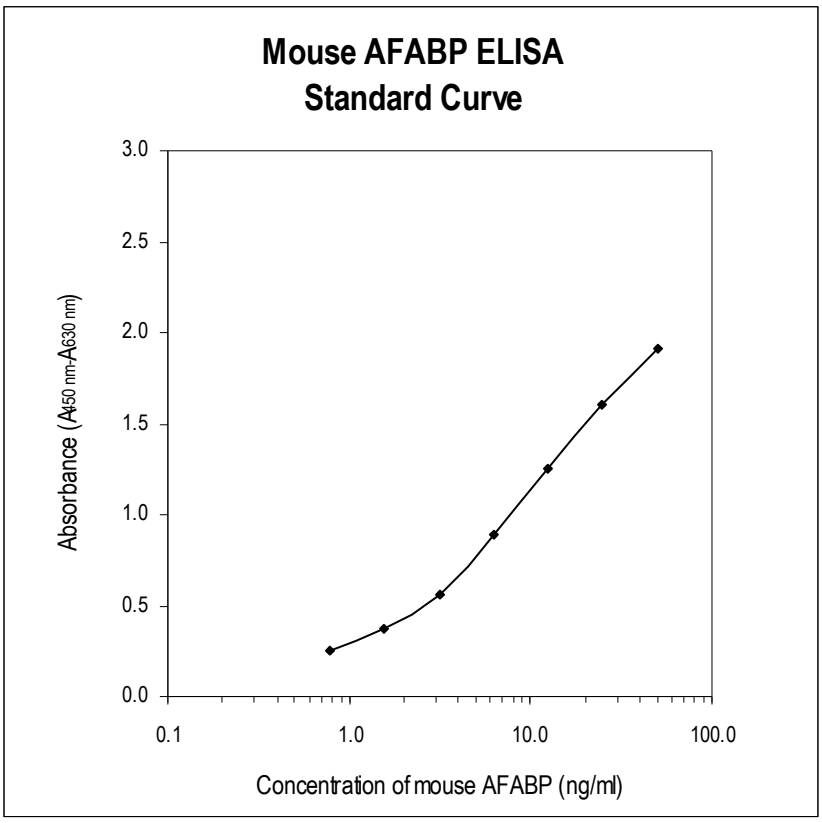


Figure 2: Typical Standard Curve for Mouse AFABP ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Mouse AFABP 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 mouse AFABP values in wells and is 0.23 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

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

- **Specificity**

The antibodies used in this ELISA are specific for mouse AFABP with no detectable crossreactivities to mouse/rat leptin, adiponectin, resistin at 50 ng/ml.

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
Human	no
Horse	no
Monkey	no
Pig	no
Rabbit	no
Rat	yes
Sheep	no

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

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	221	13.9	6.3
2	576	63.1	11.0

Inter-assay (Run-to-Run) (n=4)

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	223	11.8	5.3
2	1 313	117.5	8.9

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	790	-	-
	2x	393	395	99.4
	4x	198	197	101.1
	8x	101	99	102.2
2	-	1 436	-	-
	2x	730	718	101.6
	4x	381	359	106.2
	8x	173	180	96.3

- **Stability of samples**

Samples should be stored at –20°C. However, no decline in concentration of mouse AFABP was observed in serum 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.

- **Effect of Freezing/Thawing**

No decline was observed in concentration of mouse AFABP in serum 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 value (ng/ml)*
1	1x	204.3
	3x	187.8
	5x	169.0
2	1x	484.8
	3x	467.0
	5x	464.4
3	1x	613.5
	3x	618.4
	5x	584.6
4	1x	1 16.8
	3x	1 23.8
	5x	1 07.5

14. DEFINITION OF THE STANDARD

The Standard used in the kit is a serum-based protein.

15. PRELIMINARY POPULATION DATA

- **Normal value and normal range in mouse serum**

Group definition: mouse sera taken from 146 random selected BalbC, 2-10 months old
Normal value (mean +/- SEM) = 672 +/- 36 ng/ml
Normal range (mean +/- 2 SD) = 672 +/- 882 ng/ml

- **Reference range**

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 reference ranges for mouse AFABP levels with the assay.

16. METHOD COMPARISON

BioVendor Mouse AFABP ELISA has not been compared to any other 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 or samples

18. REFERENCES

References to mouse adipocyte:

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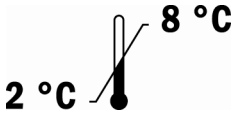
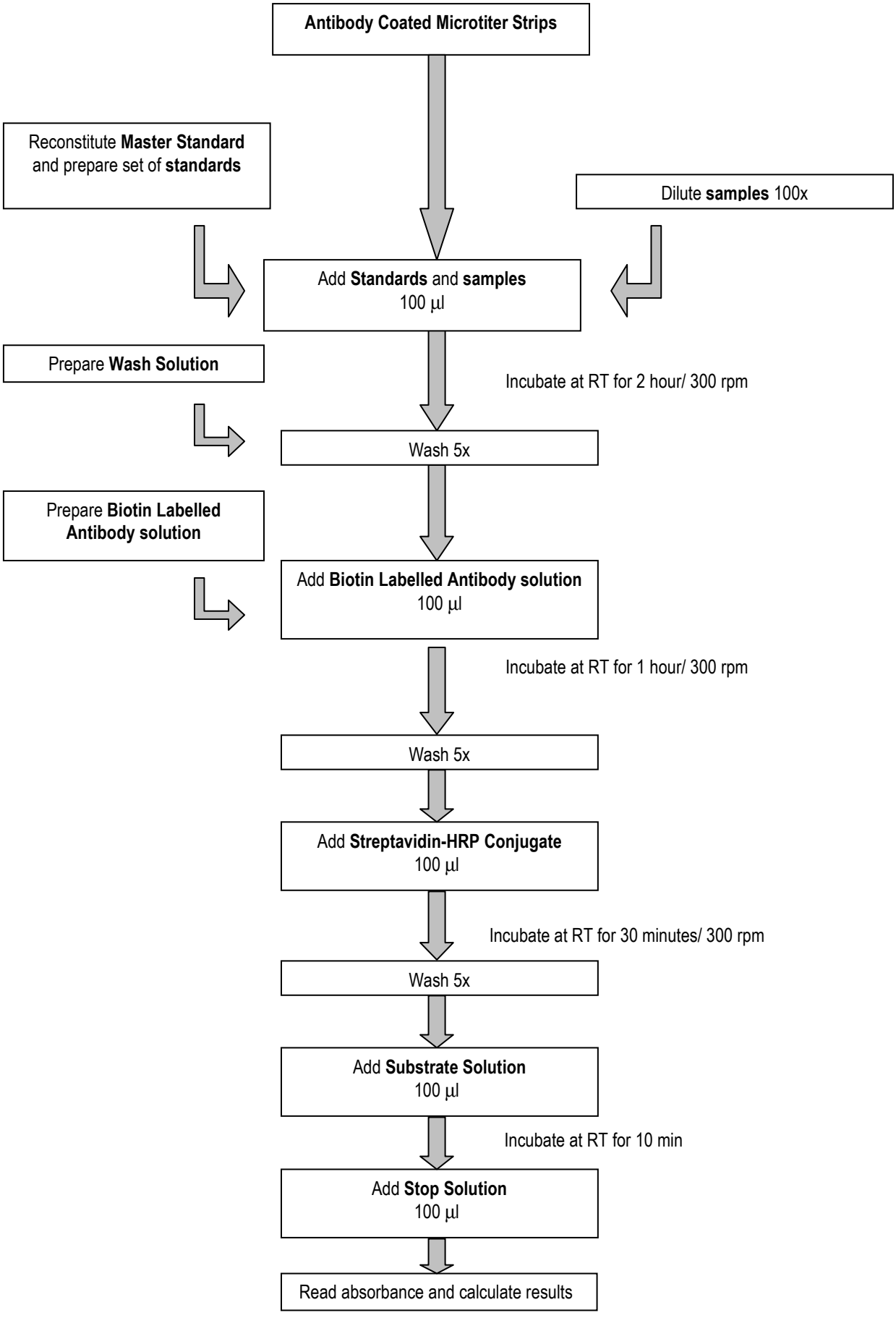
» For more references on this product see our WebPages at www.biovendor.com

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



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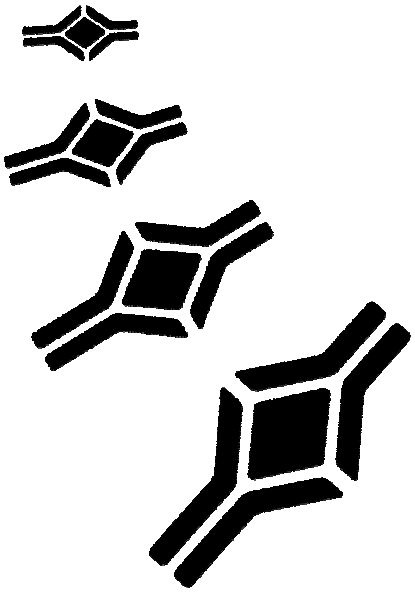

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