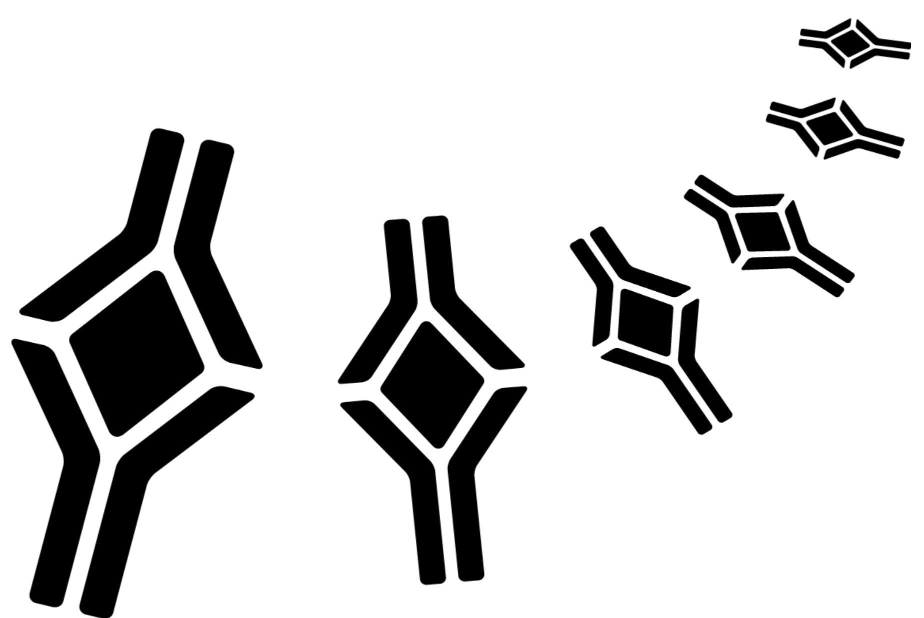

HUMAN EPIDERMAL FABP ELISA

Product Data Sheet

Cat. No.: RD191060200R

For Research Use Only

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1. INTRODED USE 3

1. INTENDED USE

The RD191060200R Human Epidermal FABP ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human EFABP in serum, plasma, urine and breast milk.

Features

- **For research use only!**
- The total assay time is less than 3 hours
- The kit measures total EFABP in serum, plasma (EDTA, heparin), urine and breast milk
- Assay format is 96 wells
- Standard is recombinant protein
- 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

Human epidermal fatty acid binding protein (EFABP, FABP5, in mice also called mal1) is a 15 kD member of the fatty acid binding protein family, which is known for the ability to bind fatty acids and related compounds (bile acids and retinoids). The epidermal fatty acid binding protein and adipocyte fatty acid binding protein (AFABP, FABP4) are closely related and both are expressed in adipocytes. Absence of EFABP/mal1 resulted in increased systemic insulin sensitivity in models of obesity and insulin resistance. Adipocytes isolated from mal1-deficient mice also exhibited enhanced insulin-stimulated glucose transport capacity. In contrast, mice expressing high levels of mal1 in adipose tissue display reduced systemic insulin sensitivity.

Areas of investigation:
Energy metabolism and body weight regulation
Type 2 diabetes mellitus
Cardiovascular disease
Oncology

4. TEST PRINCIPLE

In the BioVendor Human Epidermal FABP ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human EFABP antibody. After 60 minutes incubation followed by washing, biotin-labelled polyclonal anti-human EFABP antibody is added and incubated with the captured EFABP 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. The absorbance is proportional to the concentration of EFABP. 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 may contain components of human or 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	2 vials
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Biotin-Ab Diluent	ready to use	13 ml
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
- 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 desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate
Biotin-Ab Diluent
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:**

Human EFABP Master Standard:
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of Master 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 human EFABP in the stock solution is **40 ng/ml.**

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	40 ng/ml
500 μl of stock	500 μl	20 ng/ml
500 μl of std. 20 ng/ml	500 μl	10 ng/ml
500 μl of std. 10 ng/ml	500 μl	5 ng/ml
500 μl of std. 5 ng/ml	750 μl	2 ng/ml
500 μl of std. 2 ng/ml	500 μl	1 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
Do not store the reconstituted Master Standard and/or diluted standard solutions.

Biotin Labelled Antibody:
Refer to the Certificate of Analysis for current volume of distilled water needed for reconstitution of Biotin Labelled Antibody!!!
Reconstitute the lyophilized Biotin Labelled Antibody with distilled water just prior to the assay. Let it dissolve at least 15 minutes with occasionally gently shaking (not to foam). Dilute Biotin Labelled Antibody Concentrate 100x with Biotin-Ab Diluent (e.g. 10 μl of Biotin Labelled Antibody Concentrate + 990 μl of Biotin-Ab Diluent for 8 wells).

Stability and storage:
Do not store the reconstituted and/or diluted Biotin Labelled Antibody solutions.

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution, e.g. 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 EFABP in serum, plasma (EDTA, heparin), urine and breast milk.

Samples should be assayed immediately after collection or should be stored at -20°C or -70°C. Thoroughly mix 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 serum, plasma or breast milk samples 4x with Dilution Buffer just prior to the assay, e.g. 40 μl of sample + 120 μl of Dilution Buffer for singlets, or preferably 60 μl of sample + 180 μl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.
Dilute urine samples 2x with Dilution Buffer just prior to the assay, e.g. 70 μl of sample + 70 μl of Dilution Buffer for singlets, or preferably 120 μl of sample + 120 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.

See Chapter 13 for effect of sample matrix (serum/plasma) on the concentration of EFABP.

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, Dilution Buffer (=Blank) and diluted 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 **1 hour**, 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. Pipet **100 µl** l 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 20 minutes] if the reaction temperature is below 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 EFABP 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 four times. 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 40**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	**Standard 20**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	**Standard 10**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	**Standard 5**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	**Standard 2**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	**Standard 1**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
G	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40
H	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41

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 EFABP (ng/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve (i.e. *logit* of 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. 3 ng/ml (from standard curve) x 4 (dilution factor) = 12 ng/ml.

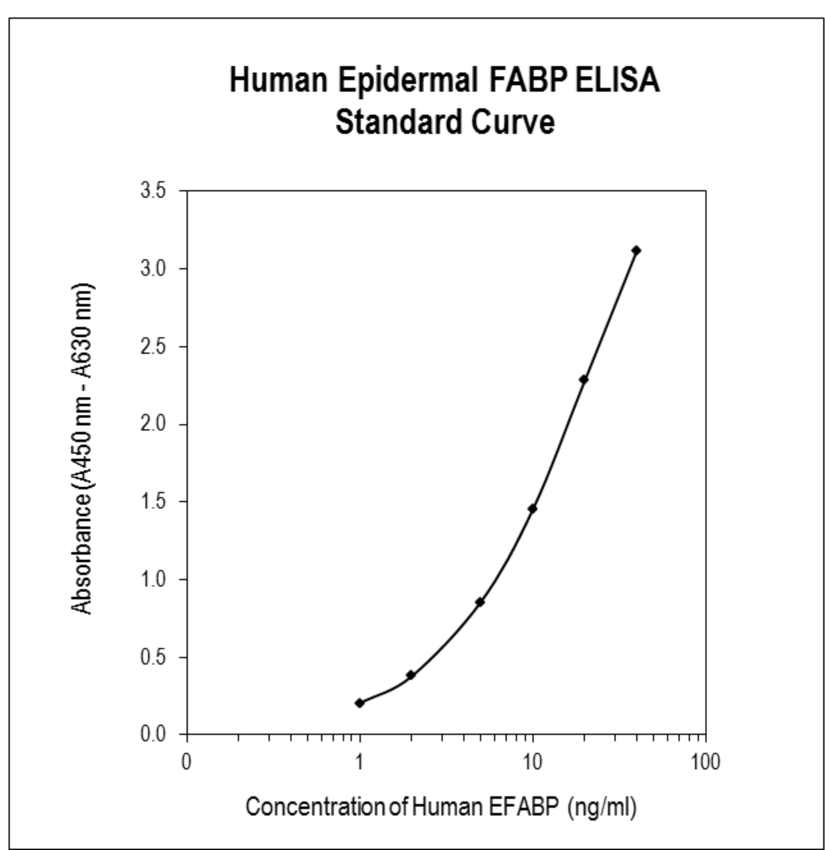

Figure 2: Typical Standard Curve for Human Epidermal FABP ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Epidermal FABP 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 EFABP values in wells and is 0.066 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

- **Specificity**

The antibodies used in this ELISA are specific for human EFABP with no detectable crossreactivities to human LFABP, IFABP, AFABP at 50 ng/ml.

Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	21.99	0.04	5.4
2	17.47	1.08	6.2

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	10.38	0.58	5.6
2	19.04	1.25	6.6

- **Spiking Recovery**

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

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	7.14 9.38 10.90 16.71	- 9.14 11.14 17.14	- 102.6 97.9 97.5
2	5.14 5.84 9.03 14.82	- 7.14 9.14 15.14	- 81.8 98.8 97.9

- **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	- 2x 4x 8x	41.43 20.24 10.42 4.71	- 20.71 10.36 5.18	- 97.7 100.6 90.9
2	- 2x 4x 8x	37.58 18.19 8.88 5.03	- 18.79 9.40 4.70	- 96.8 94.5 107.1

- **Effect of sample matrix**

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

Results are shown below:

Volunteer No.	*Serum (ng/ml)*	*Plasma (ng/ml)*		
		EDTA	*Citrate*	*Heparin*
1	3.49	3.07	6.10	5.46
2	4.93	7.35	4.45	8.34
3	4.86	6.10	10.93	7.34
4	5.45	6.73	4.43	7.82
5	6.06	7.26	4.60	7.53
6	7.27	8.53	5.69	8.77
7	3.25	3.55	3.51	6.38
8	8.49	8.72	6.77	11.15
9	3.19	5.29	3.81	6.94
10	10.42	10.97	8.84	12.84
Mean (ng/ml)	**5.74**	**6.76**	**5.91**	**8.26**
Mean Plasma/Serum (%)	**-**	**118**	**103**	**144**
Coefficient of determination R^2	**-**	**0.87**	**0.21**	**0.90**

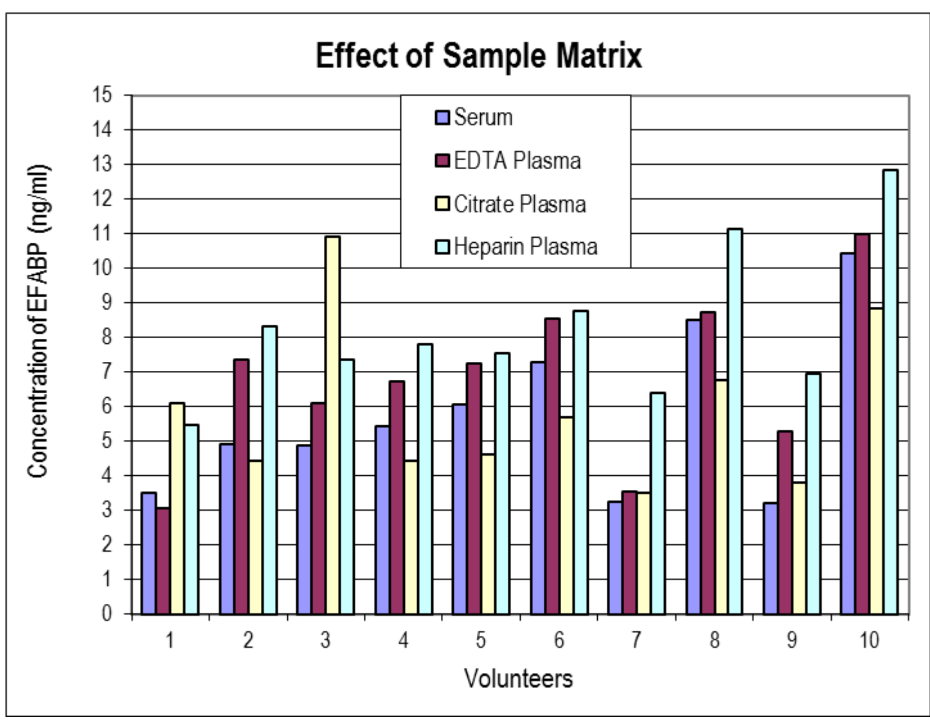

Figure 3: EFABP levels measured using Human Epidermal FABP ELISA in serum, EDTA, citrate and heparin plasma from the same 10 individuals.

14. DEFINITION OF THE STANDARD

The recombinant human protein EFABP is used as the Standard. The recombinant human EFABP, produced in *E. coli*, is 15.2 kDa protein containing 135 amino acid residues.

15. METHOD COMPARISON

The BioVendor Human Epidermal FABP ELISA has not been compared to any commercial immunoassay.

16. 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

17. REFERENCES

References to Human Epidermal FABP ELISA:

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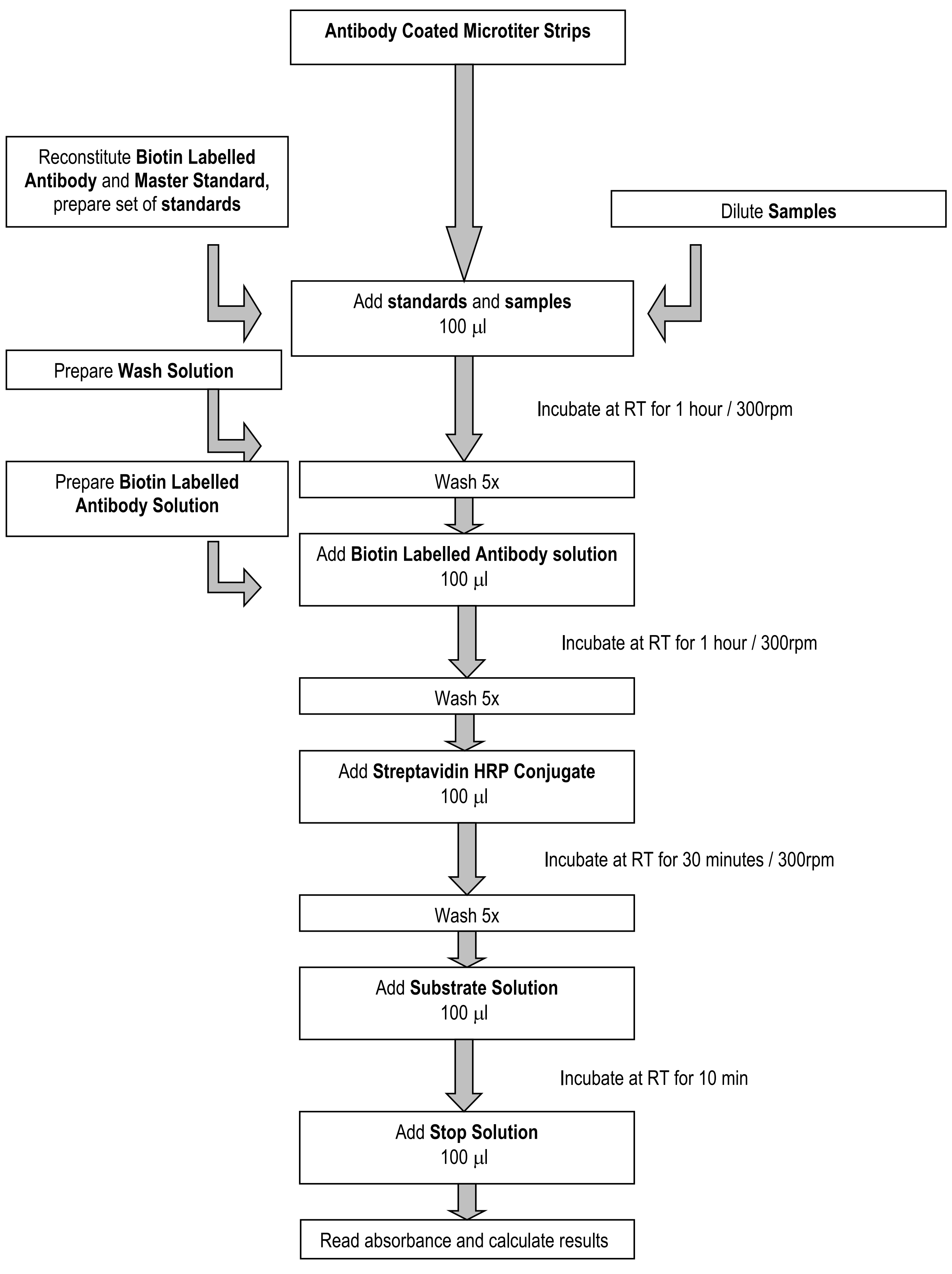
For more references on this product see our WebPages at www.biovendor.com

18. EXPLANATION OF SYMBOLS

Symbol	Explanation
REF	Catalogue number
Cont.	Content
LOT	Lot number
⚠	Attention, see instructions for use
☣	Potential biological hazard
⌛	Expiry date
2 °C – 8 °C	Storage conditions
🏭	Name and registered office of the manufacturer

Assay Procedure Summary



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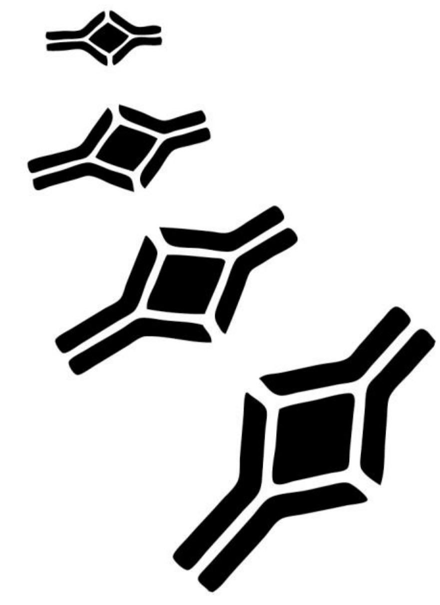

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