BioVendor Group

ENG

Instructions for Use:

HUMAN INTERLEUKIN-1 BETA ELISA

Catalogue number:

RD194559200R

For research use only!

BioVendor R&D

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HISTORY OF CHANGES

Previous version	Current version
ENG.004.A	ENG.005.A
A symbol indicating the manufacturer added.	

1. INTENDED USE

The RD194559200R Human Interleukin-1 Beta ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human IL-1 Beta.

Features

- **It is intended for research use only**
- The total assay time is less than 4 hours
- The kit measures IL-1 Beta in serum, plasma (EDTA, heparin, citrate) and saliva
- Assay format is 96 wells
- 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

IL-1 Beta is a polypeptide cytokine and represents one of the most important mediators of inflammation and host responses to infections. This protein is a member of the interleukin-1 cytokine family, which is closely linked to the innate immune response (1). Mature form of IL-1 Beta has molecular weight of 17.5 kDa and exists in monomeric form.
Expression of the gene that encodes IL-1 Beta is induced by various proinflammatory stimuli such as bacterial and viral products, other cytokines, cellular injury, monosodium urate crystals and hypoxia (2, 3). IL-1 Beta is produced by many cell types of both the peripheral and central immune system, including lymphocytes and monocytes (2).
The synthesis and release of IL-1 Beta is tightly regulated (7). IL-1B is secreted only upon inflammatory signals and is not present in homeostatic conditions (8). IL-1 Beta is synthetized as biologically inactive 35 kDa cytosolic precursor (9). Processing of bioactive IL-1 Beta depends on activation of caspase-1 by protein complexes termed the inflammasomes (10). Most of the IL-1 Beta remains intracellular;an additional signal such as ATP is needed for its secretion (8, 10).
Even low concentrations of IL-1 Beta cause fever, hypotension and production of additional proinflammatory chemokines/cytokines, such as IL-6 (4). IL-1 Beta exerts biological effects by binding the membrane-bound type I IL-1 receptor (IL-1R), which then associates with the IL-1-receptor accessory protein (IL-1RAcP) to form a complex capable of intracellular signaling (5). This signalling controls expression of a number of inflammatory and catabolic genes. (6)
Besides its favorable role in mediating host responses to microbial invasion, IL-1 Beta has also harmful effects (11). IL-1 Beta can promote tumor invasiveness, tumor angiogenesis and metastasis (8). IL-1 Beta also exacerbates damage during chronic diseases and acute tissue injury (12). Overexpression of IL-1 Beta was observed in the pathophysiological changes that occur during different diseases, such as rheumatoid arthritis, neuropathic pain, inflammatory bowel disease, osteoarthritis, multiple sclerosis and neurodegenerative diseases (13, 14, 15).
It was observed that IL-1 Beta impairs insulin-producing Beta-cell function (16). Macrophage-derived IL-1 Beta production in insulin-sensitive organs leads to progression of inflammation and induction of insulin resistance in obesity (17).
Regarding other biofluids, it was found that IL-1 Beta is one of the most abundant cytokine in saliva. It was observed that salivary level of IL-1 Beta was higher in the patients with periodontitis compared to periodontally healthy subjects (18, 19).

Areas of investigation:
Cytokines
Immune Response, Infection and Inflammation
Oncology
Sepsis

4. TEST PRINCIPLE

In the BioVendor Human Interleukin-1 Beta ELISA, standards and samples are incubated in a microplate wells pre-coated with monoclonal anti-human IL-1 Beta antibody. After 60 minutes incubation and a washing, biotin-labelled monoclonal anti-human IL-1 Beta antibody is added and incubated with captured IL-1 Beta for 60 minutes. After another washing, the 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. The absorbance is proportional to the concentration of IL-1 Beta. A standard curve is constructed by plotting absorbance values against concentrations of IL-1 Beta 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 Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml

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, 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°C-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°C-8°C.

Assay reagents supplied concentrated or lyophilized:

Human IL-1 Beta 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 (do not foam).
The resulting concentration of IL-1 Beta in the stock solution is **80 pg/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	–	80 pg/ml
250 µl of stock	250 µl	40 pg/ml
250 µl of 40 pg/ml	250 µl	20 pg/ml
250 µl of 20 pg/ml	250 µl	10 pg/ml
250 µl of 10 pg/ml	250 µl	5 pg/ml
250 µl of 5 pg/ml	250 µl	2.5 pg/ml
250 µl of 2.5 pg/ml	250 µl	1.25 pg/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 Biotin-Ab Diluent needed for reconstitution of Biotin Labelled Antibody!!!
Reconstitute the lyophilized Biotin Labelled Antibody with Biotin-Ab Diluent just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (do not 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 strip (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°C-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2°C-8°C.

10. PREPARATION OF SAMPLES

The kit measures human IL-1 Beta in serum, plasma (EDTA, citrate, heparin) and saliva.

Serum and plasma samples

Samples can be assayed immediately after collection, or should be stored frozen. 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. **Separate the serum or plasma from the cells within two hours**.

An appropriate dilution should be assessed by the researcher in advance to batch measurement. Recommended starting dilution is indicated below.
Dilute samples **3x** with Dilution Buffer just prior to the assay. e.g. 50 µl of sample + 100 µl of Dilution Buffer for singlets, or preferably 80 µl of sample + 160 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Saliva samples:

Collect saliva into a clean tube without force or inducement and before eating, drinking or brushing the teeth. Before saliva collection, rinse the mouth with water. Avoid using blood-contaminated specimens. Store samples at -70°C. Just prior to the assay, centrifuge thawed samples at 13,000 rpm for 5 min. Collect supernatants into labelled tubes.
Dilute saliva supernatant samples **20x** with the Dilution Buffer just prior to the assay, e.g. 15 µl of sample + 285 µl of Dilution Buffer.

Stability and storage:
Samples should be stored at -20°C, or preferably at -70°C for long-term storage (for up to one year). Avoid repeated freeze/thaw cycles.
Do not store the diluted samples.

Note: It is recommended to use a precise 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 **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 a paper towel.
4. Pipet **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 a paper towel.
7. Pipet **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 a 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 less 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 1: 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 IL-1 Beta 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 two 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 80**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 40**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 20**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 10**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	**Standard 5**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	**Standard 2.5**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	**Standard 1.25**	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 IL-1 Beta (pg/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. 2.2 pg/ml (from standard curve) x 3 (dilution factor) = 6.6 pg/ml.

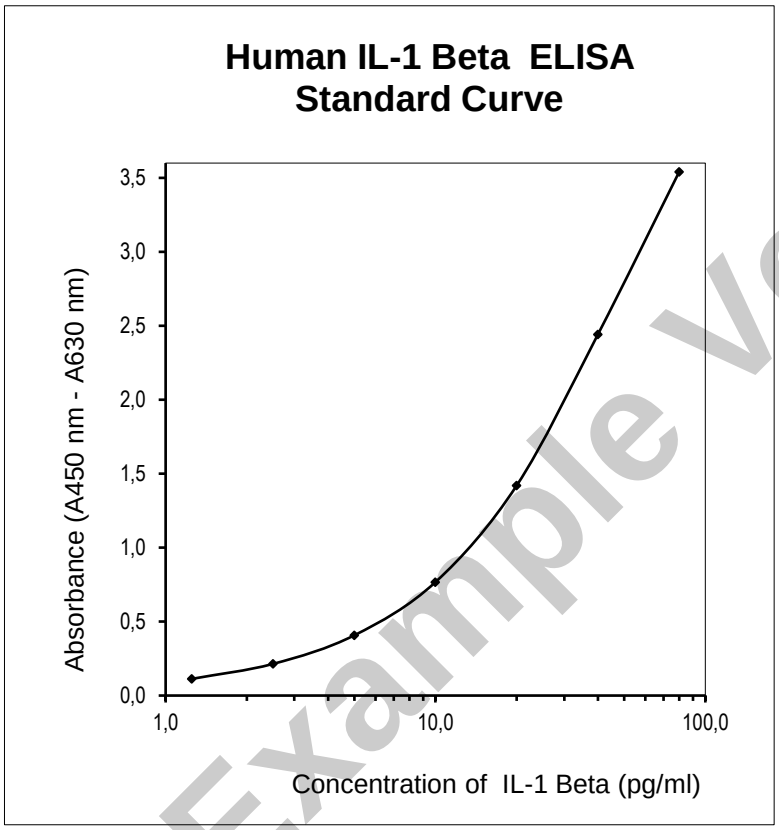

Figure 2: Typical Standard Curve for Human IL-1 Beta ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human IL-1 Beta 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 IL-1 Beta values in wells and is 0.4 pg/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.

Presented results are multiplied by respective dilution factor

Precision

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

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
Saliva 1	168.77	4.5	2.7
Saliva 2	45.67	1.9	4.2

Inter-assay (Run-to-Run) (n=5)

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
Serum 1	54.93	2.8	5.2
Serum 2	101.57	6.8	6.7

Spiking Recovery

Samples were spiked with different amounts of recombinant human IL-1 Beta protein and assayed.

Sample	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Serum 1	2.36	-	-
	8.09	9.86	82.0
	14.26	17.36	82.1
	26.77	32.36	82.7
Serum 2	3.24	-	-
	9.44	10.74	87.9
	14.87	18.24	81.5
	26.92	33.24	81.0

Sample	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Saliva 1	139.59	-	-
	200.98	189.59	106.0
	248.48	239.59	103.7
	371.43	339.59	109.4
Saliva 2	74.18	-	-
	128.06	124.18	103.1
	164.94	174.18	94.7
	287.52	274.18	104.9

Linearity

Samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Serum 1	-	79.66	-	-
	2x	40.05	39.83	100.6
	4x	20.34	19.91	102.1
	8x	9.43	9.96	94.7
Serum 2	-	56.22	-	-
	2x	28.56	28.11	101.6
	4x	14.99	14.04	106.7
	8x	7.64	7.03	108.7

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Saliva 1	-	1080.81	-	-
	2x	506.27	540.41	93.7
	4x	264.56	270.20	97.9
	8x	140.78	135.10	104.2
Saliva 2	-	663.22	-	-
	2x	307.99	331.61	92.9
	4x	153.41	165.81	92.5
	8x	80.40	82.90	97.0

14. DEFINITION OF THE STANDARD

Recombinant human IL-1 Beta is used as the standard. The recombinant IL-1 Beta produced in *E. coli* is a 17.3 kDa protein consisting of 153 amino acid residues.

15. METHOD COMPARISON

The BioVendor Human IL-1 Beta ELISA was compared to another commercial immunoassay by measuring 23 saliva samples. The following correlation graph was obtained:

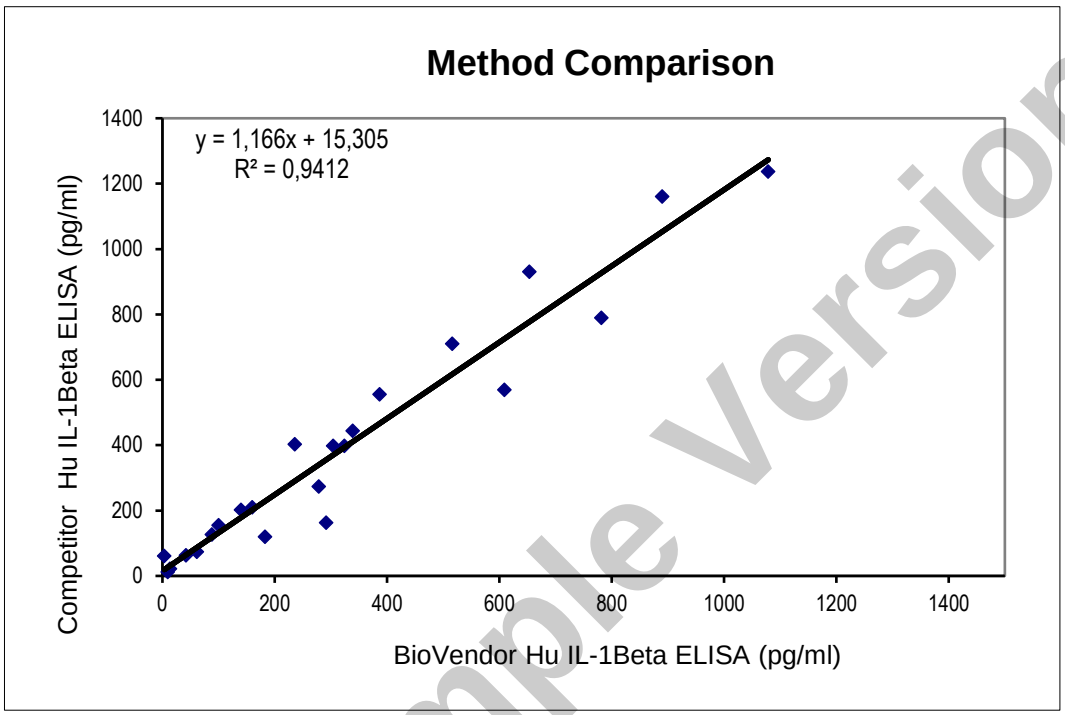

Figure 3: Method comparison

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
- Manual washing
- 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

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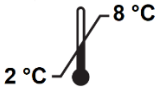
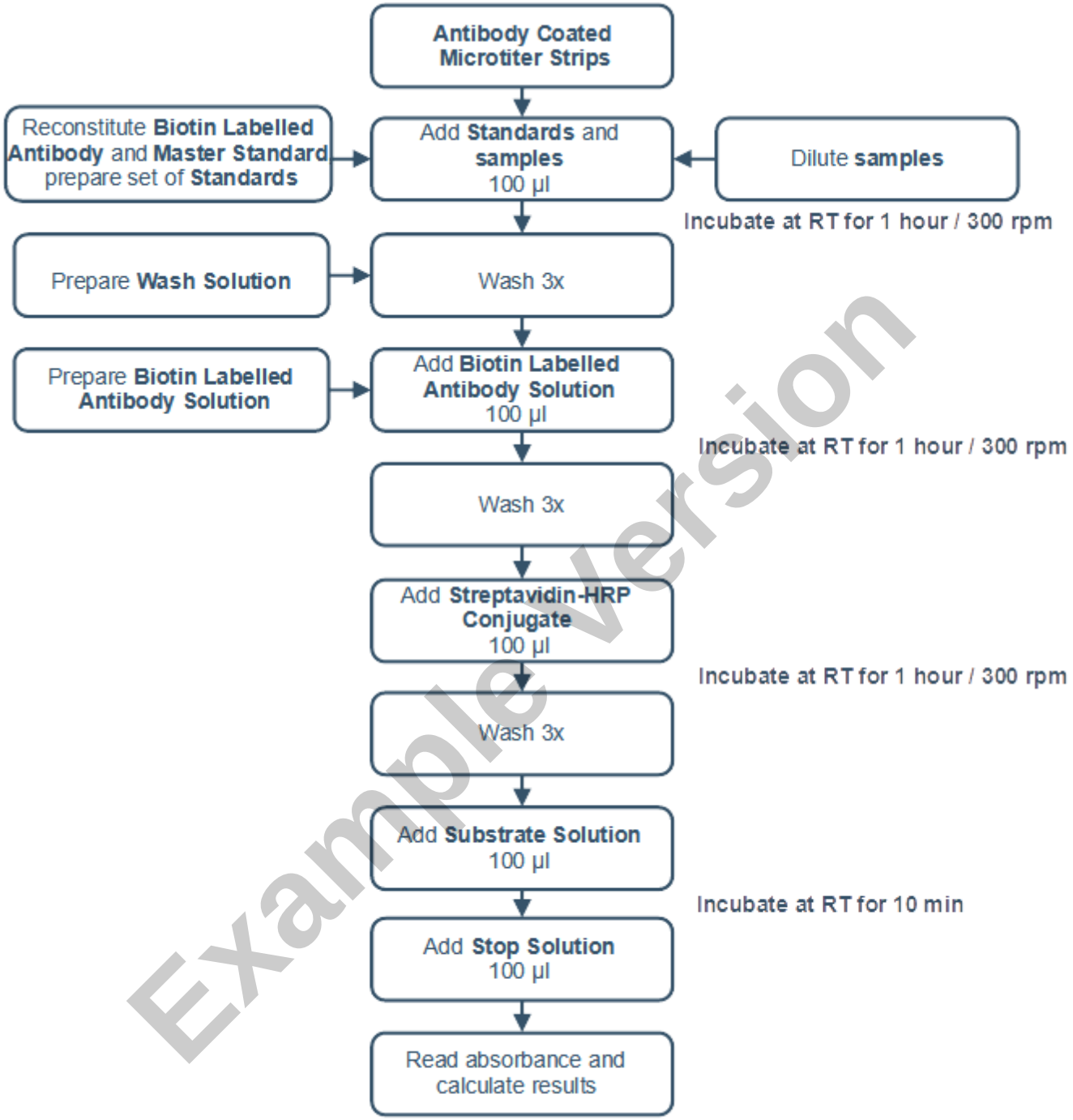
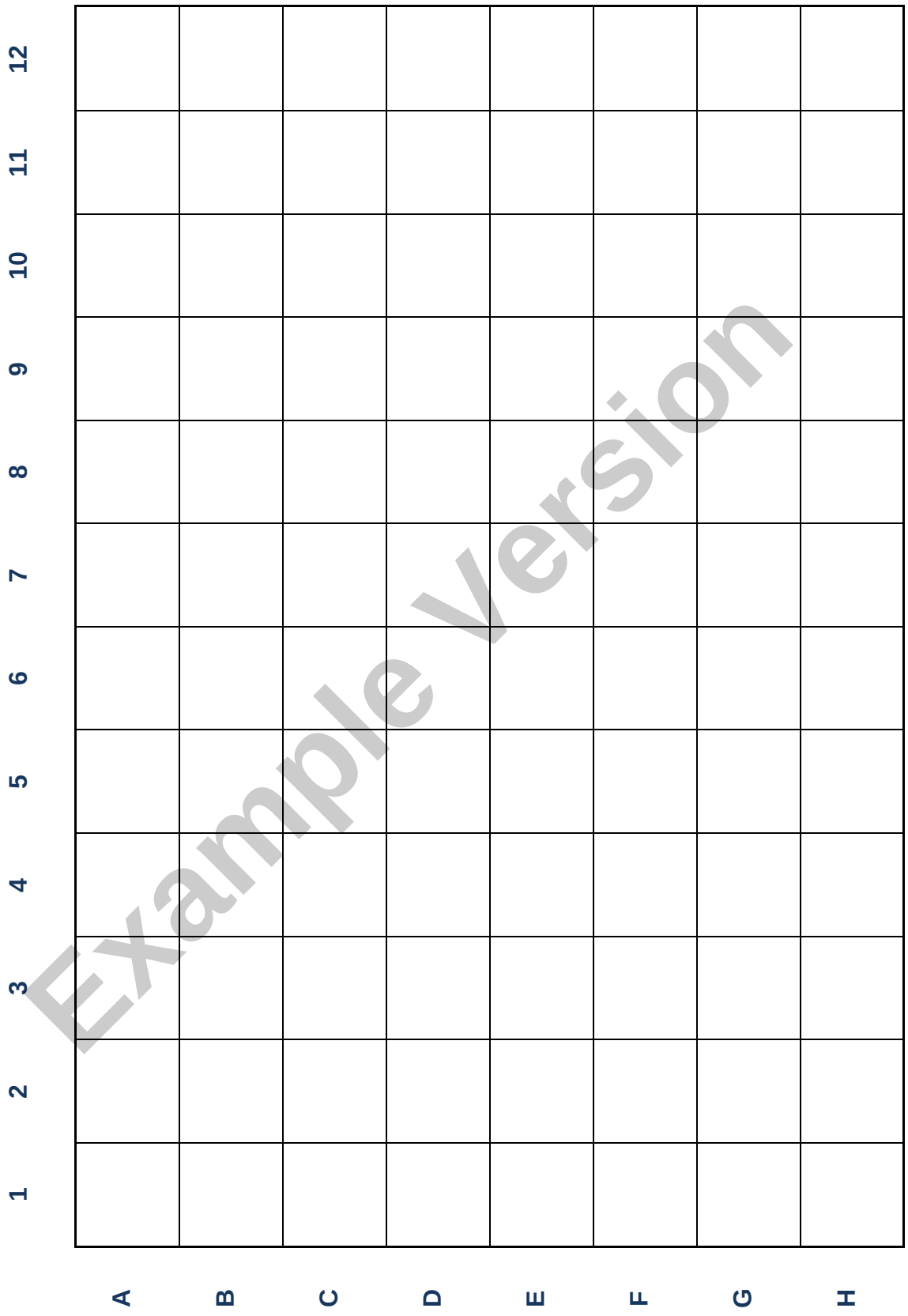
For more references on this product see our web pages at www.biovendor.com.

18. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks

19. ASSAY PROCEDURE - SUMMARY





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