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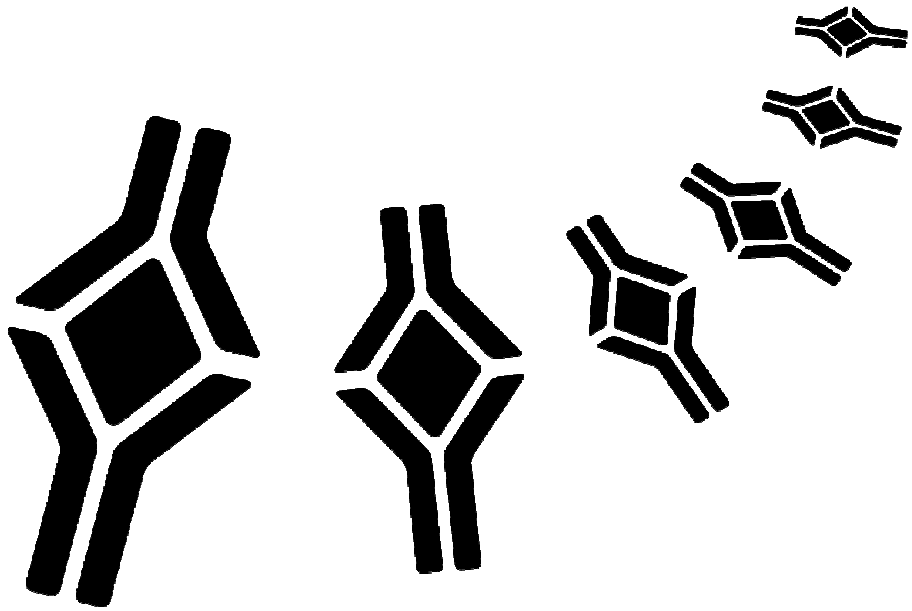
Research
and Diagnostic Products



HUMAN VILIP-1 ELISA

Product Data Sheet

Cat. No.: RD191119200R

For Research Use Only

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 RD191119200R Human VILIP-1 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human VILIP-1(Visinin-like protein 1).

Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures human VILIP-1 in serum, plasma, CSF and tissue extracts
- 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

Visinin like protein 1 (VILIP-1, VLP-1 or VSNL-1) is a cytoplasmic protein of low molecular weight (approximately 22 kDa) consisting of 191 amino acid residues. It belongs to the visinin/recoverin subfamily of neuronal calcium sensor proteins involved in calcium-dependent signal transduction mechanisms in neurons. It is found primarily in the brain, in nerve cells, but it also has a peripheral distribution in liver, lung, kidney, spleen, pancreas and colon. When localized at the membrane, it modulates various cellular signal transduction pathways, including cyclic adenosine monophosphate (cAMP)- and cyclic guanosine monophosphate (cGMP)-signaling in neural cells, human embryonic kidney cells, the pancreatic β cell line MIN6, and various skin tumor cell lines. It contains four internal repeats of 36-38 amino-acids, each containing a potential EF-hand domain. Two of the four EF-hand Ca2+-binding motifs of VILIP-1 are able to bind either Ca^{2+} or Mg^{2+}in a non-cooperative manner. Binding of Ca^{2+} leads to specific conformational changes in the protein and this may regulate the interaction of VILIP with intracellular target molecules.
VILIP-1 has been identified as a potential biomarker for brain injury and several neurodegenerative diseases. VILIP-1-expressing cells appear to be vulnerable to neurotoxic insults. As a result, the protein is released into the cerebrospinal fluid (CSF), and can be used as a biomarker for stroke and Alzheimer's disease. The intracellular protein was detected in cerebrospinal fluid (CSF) of a rat model of stroke and in plasma of patients after stroke. VILIP-1 was detected in 44% of subjects with stroke, in samples taken 24 hours after onset of stroke, and in 8% of controls with no stroke. In Alzheimer's disease, CSF levels of VILIP-1 were significantly higher than in healthy individuals. Post mortem studies in the hippocampus of schizophrenia patients revealed increased expression of VILIP-1 in interneurons, while its expression in pyramidal neurons was downregulated. Expressions of VILIP-1 were also found in different types of cancer and in pancreatic α- and β-cells, being involved in the regulation of insulin secretion and insulin gene expression.

<u>Areas of investigation:</u>
Neurodegenerative disease
Brain injury
Insulin secretion
Cancer

4. TEST PRINCIPLE

In the BioVendor Human VILIP-1 ELISA, the standards, quality controls and samples are incubated in microtitrate wells pre-coated with polyclonal anti-human VILIP-1 antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human VILIP-1 antibody is added and incubated with captured VILIP-1 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. The absorbance is proportional to the concentration of VILIP-1. 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 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

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (100x)	concentrated	0.14 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Biotin Ab - Diluent	ready to use	13 ml
Dilution Buffer	ready to use	13 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Instruction Manual + 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
Substrate Solution
Stop Solution
Dilution Buffer
Stability and storage:
Opened reagents are stable 3 months when stored at 2-8°C.

- **Assay reagents supplied concentrated or lyophilized:**

Human VILIP-1 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 human VILIP-1 in the stock solution is **5 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	5 ng/ml
250 µl of 5 ng/ml	250 µl	2.5 ng/ml
200 µl of 2.5 ng/ml	300 µl	1 ng/ml
250 µl of 1 ng/ml	250 µl	0.5 ng/ml
250 µl of 0.5 ng/ml	250 µl	0.25 ng/ml
200 µl of 0.25 ng/ml	300 µl	0.1 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
The reconstituted standard stock solution (5 ng/ml) must be used immediately or aliquoted and stored frozen at –20 °C for 3 months. Avoid repeated freeze/thaw cycles.
Do not store the diluted Standard solutions.

Quality Controls HIGH, LOW
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
The 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.
Note:
Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

Biotin Labelled Antibody Conc. (100x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) with 99 parts Biotin-Ab Diluent. Example: 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Biotin-Ab Diluent for 1 strip (8 wells).
Stability and storage:
Opened Biotin Labelled Antibody Concentrate (100x) is stable 3 months when stored at 2-8°C.
Do not store diluted Biotin Labelled Antibody solution.

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 human VILIP-1 in serum, plasma, CSF and tissue extracts.

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 serum, plasma and CSF samples 3x with Dilution Buffer just prior to the assay (e.g. 50 µl of sample + 100 µl of Dilution Buffer when assaying samples as singlets or preferably 100 µl of sample + 200 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 stability of samples when stored at 2-8°C and effect of freezing/thawing on the concentration of VILIP-1.

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 info@biovendor.com if assaying tissue extract samples.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of Standards, Quality Controls, 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. 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 **1 hour**, 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. (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). No shaking!
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 VILIP-1 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 5**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 2.5**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 1**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 0.5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 0.25**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 0.1**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**QC HIGH**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC LOW**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microtiter plate 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 VILIP-1 (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. 0.301 ng/ml (from standard curve) x 3 (dilution factor) = 0.903 ng/ml.

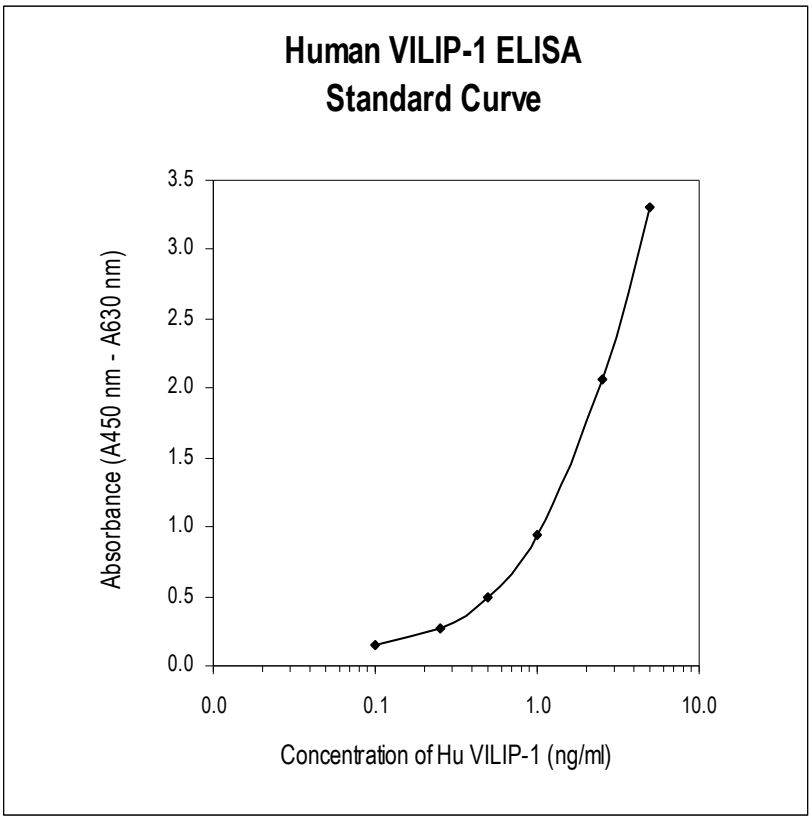


Figure 2: Typical Standard Curve for Human VILIP-1 ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human VILIP-1 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 VILIP-1 values in wells and is 0.027 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding VILIP-1 level of 5 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the VILIP-1 concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human VILIP-1.

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	yes
Dog	no
Goat	yes
Hamster	no
Horse	no
Monkey	no
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

» **Presented results are multiplied by respective dilution factor**
» **For the next subchapters, serum samples containing native protein from brain tissue extract were used**

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	2.85	0.13	4.43
2	2 112.50	131.51	6.23

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	2.95	0.13	4.45
2	2 213.45	44.09	1.99

- **Spiking Recovery**

Samples were spiked with different amounts of human VILIP-1 and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	1.91	-	-
	2.20	2.16	101.7
	2.68	2.57	104.0
	3.44	3.39	101.6
2	2 842.7	-	-
	3 319.4	3 178.7	104.4
	3 996.4	3 726.7	107.2
	5 104.5	4 814.7	106.0

- **Linearity**

Samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	2 231.9	-	-
	2x	1 092.7	1 116.0	97.9
	4x	488.8	558.0	87.6
	8x	260.4	279.0	93.3
2	-	5.15	-	-
	2x	2.42	2.58	94.1
	4x	1.22	1.29	94.6
	8x	0.63	0.64	98.2

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

Samples should be stored at –20°C. However, no decline in concentration of VILIP-1 was observed in samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and thimerosal, resulting in the final concentration of 0.03% and 0.05%, respectively.

Sample	*Incubation Temp, Period*	*Sample (ng/ml)*
1	-20°C	5.06
	2-8°C, 1 day	5.00
	2-8°C, 7 days	5.14
2	-20°C	3.73
	2-8°C, 1 day	4.00
	2-8°C, 7 days	3.92
3	-20°C	3.51
	2-8°C, 1 day	3.28
	2-8°C, 7 days	3.14

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human VILIP-1 in 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*	*Sample (ng/ml)*
1	1x	4.85
	3x	4.95
	5x	5.18
2	1x	3.83
	3x	3.96
	5x	4.00
3	1x	3.28
	3x	3.39
	5x	3.59

- **Reference ranges**

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 VILIP-1 levels with the assay.

14. DEFINITION OF THE STANDARD

In this assay, the recombinant protein is used as a Standard. The recombinant VILIP-1 is a 201 amino acid residues protein produced in *E. coli*. The calculated molecular weight is 23.4 kDa.

15. METHOD COMPARISON

The BioVendor Human VILIP-1 ELISA has not been compared to any other 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, Quality Controls or samples

17. REFERENCES

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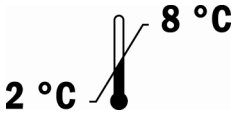
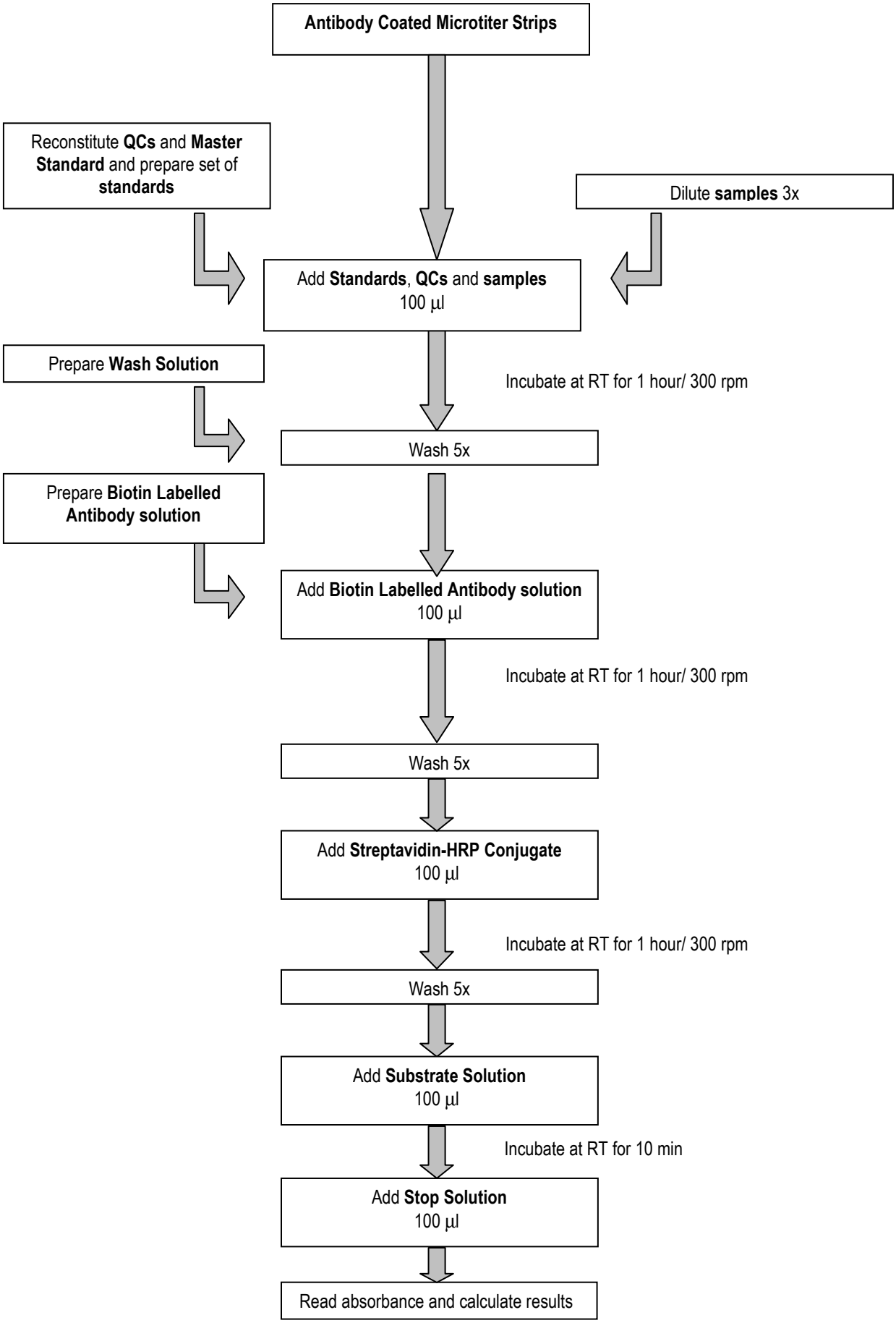
- I Schnurra, HG Bernstein, P Riederer, KH Braunewell: The neuronal calcium sensor protein VILIP-1 is associated with amyloid plaques and extracellular tangles in Alzheimer's disease and promotes cell death and tau phosphorylation in vitro: a link between calcium sensors and Alzheimer's disease?. Neurobiol Dis. 8(5):900-9

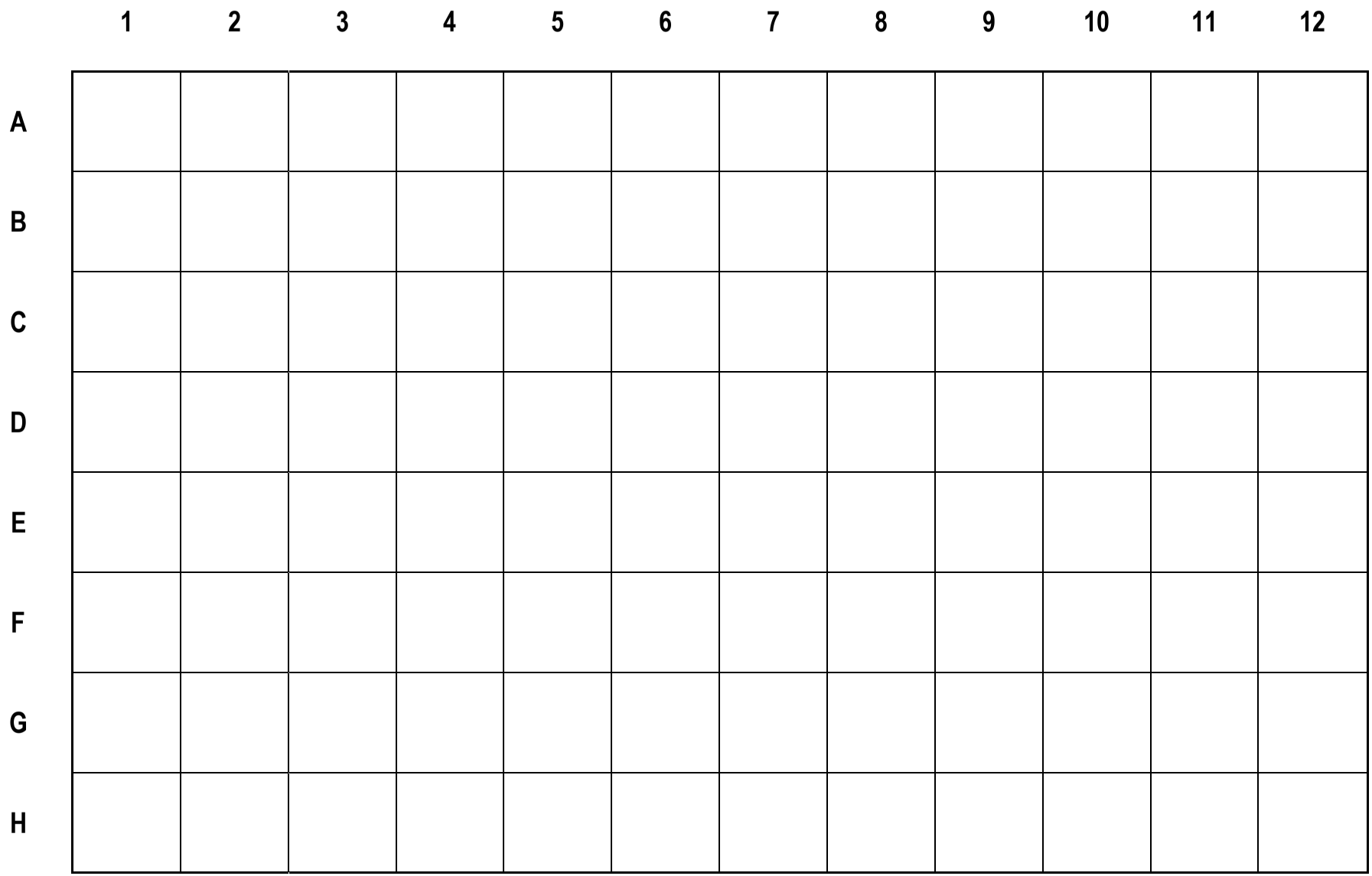
» For more references on this product see our WebPages at www.biovendor.com

18. 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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NOTES

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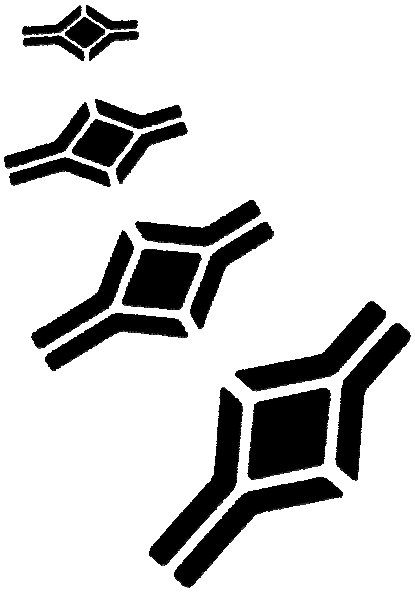

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