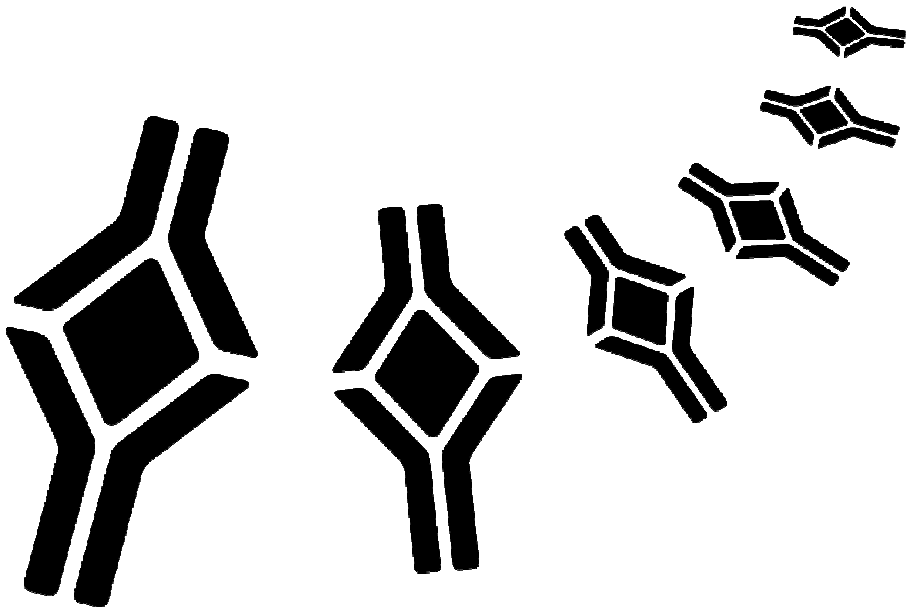

HUMAN MIDKINE ELISA

Product Data Sheet

Cat. No.: RD191042200R

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 RD191042200R Human Midkine ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human midkine.

Features

- **It is intended for research use only**
- The total assay time is less than 5 hours
- The kit measures midkine in serum, citrate plasma and heparin plasma
- Assay format is 96 wells
- Quality Control and Master Standard are human serum based
- Standard is recombinant protein based
- Standard Diluent contains animal serum
- 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

Midkine (MK, also called neurite growth promoting factor 2, NEGF-2), a product of a retinoic acid responsive gene, is a secreted 13 kDa protein belonging to the family of heparin binding growth/differentiation factors. MK shares 45% sequence identity with another member of this family called Pleiotrophin (HB-GAM). Midkine is composed of two domains held together by disulfide linkages. The C-terminally located domain contains two heparin binding sites and is usually responsible for midkine activity. Part of the MK activity is enhanced by dimerization of MK.
Midkine has been found in vertebrates from human to zebrafish and is most strongly expressed in midgestation. In the adult MK expression is restricted. In addition to normal development, MK is also involved in the pathogenesis of diseases, e.g. inflammatory diseases, human carcinomas such as esophageal, stomach, colon, pancreatic, thyroid, lung, urinary, hepatocellular, neuroblastoma, glioblastoma, Wilm´s tumor etc. High MK levels are associated with poor prognosis in some types of cancer. The increased expression in many carcinomas indicates that MK can be applied to the diagnosis of malignancy. Midkine is expressed during the reparative stage of bone fractures, also supresses infection of cells by some viruses including HIV. Anti-apoptotic and cell protecting activity of midkine makes it promising in therapy.

Areas of investigation:
Oncology
Inflammatory diseases
Preservation and repair of injured tissues

4. TEST PRINCIPLE

In the BioVendor Human Midkine ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human midkine antibody. After 120 minutes incubation at 37°C and washing, biotin labelled polyclonal anti-human midkine antibody is added and incubated with captured midkine for 60 minutes. After another washing, streptavidin–HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining HRP 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 midkine. 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
- 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	ready to use	13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control	lyophilized	1 vial
Dilution Buffer	ready to use	13 ml
Standard Diluent	ready to use	9 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
- 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.

Biotin Labelled Antibody
Streptavidin-HRP Conjugate
Standard 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:**

Master Standard
Refer to the Certificate of Analysis for current volume of Standard Diluent needed for reconstitution of Master Standard!!!
Reconstitute the lyophilized Master Standard with Standard Diluent just prior to the assay. Let it dissolve at least 30 minutes with occasional gentle shaking (not to foam). The resulting concentration of the human midkine in the stock solution is **100 ng/ml**.

Prepare set of standards using Standard Diluent as follows:

Volume of Standard	*Standard Diluent*	*Concentration*
Stock	-	100 ng/ml
100 µl of stock	900 µl	10 ng/ml
150 µl of 10 ng/ml	150 µl	5 ng/ml
150 µl of 5 ng/ml	225 µl	2 ng/ml
150 µl of 2 ng/ml	150 µl	1 ng/ml
150 µl of 1 ng/ml	150 µl	0.5 ng/ml
150 µl of 0.5 ng/ml	225 µl	0.2 ng/ml

Dilute prepared Standards 5x with Standard Diluent prior to the assay, e.g. 50 µl of Standards + 200 µl of Standard Diluent for duplicates.

Stability and storage:

Standards should be prepared from freshly reconstituted Master Standard.

Do not store the diluted Standard solutions.

Quality Control

Refer to the Certificate of Analysis for current volume of distilled water needed for reconstitution and for current Quality Control concentration!!!

Reconstitute Quality Control with distilled water just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Dilute Quality Control prior to the assay 5x with Dilution Buffer, e.g. 25 µl of Quality Control + 100 µl of Dilution Buffer for singlets, or preferably 50 µl of Quality Control + 200 µl of Dilution Buffer for duplicates.

Stability and storage:

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

Do not store the diluted Quality Control.

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 Conc. (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures midkine in serum, citrate plasma and heparin plasma

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 5x with Dilution Buffer just prior to the assay, e.g. 25 µl of sample + 100 µl of Dilution Buffer for singlets, or preferably 50 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 serum samples when stored at 2-8°C and effect of freezing/thawing on the concentration of midkine.

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 extracts.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards, Quality Control, 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 **37°C** for **2 hours**, no shaking.
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 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 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 midkine 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 10**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 5**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 2**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 1**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	**Standard 0.5**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	**Standard 0.2**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	**Blank**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
H	**Quality Control**	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 midkine 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.

Samples, Quality Control and Standards are all diluted 5x prior to analysis, so there is no need to take this dilution factor into account.

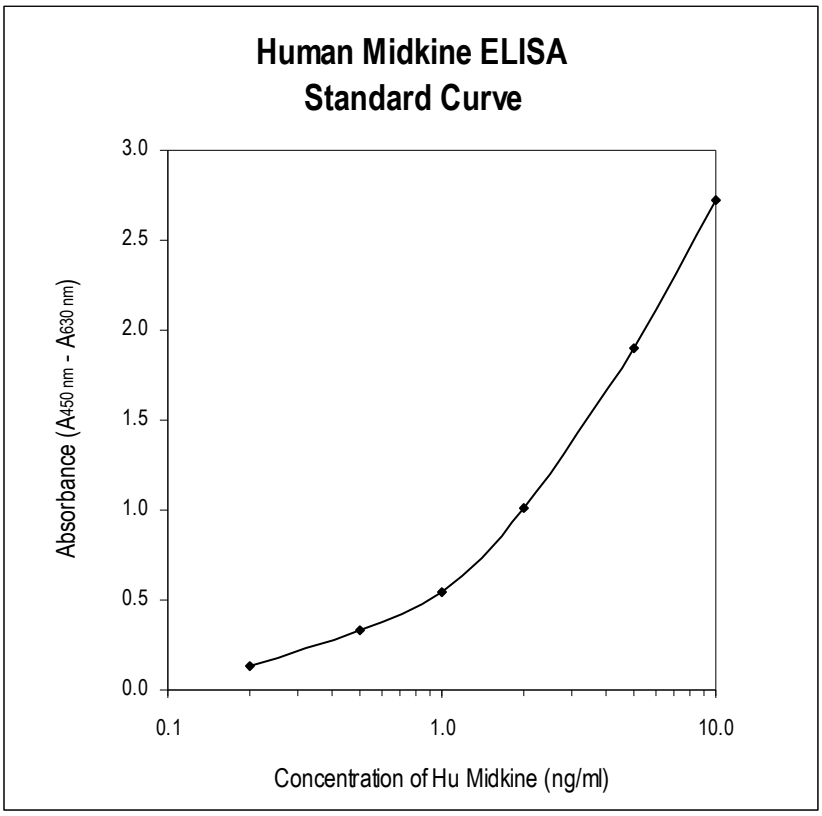

Figure 2: Typical Standard curve for Human Midkine ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Midkine 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 midkine values in wells and is 0.033 ng/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding midkine level of 10 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the midkine concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human midkine with no detectable crossreactivity to human pleiotrophin at 100 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	yes
Goat	no
Hamster	no
Horse	no
Monkey	no
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	1.27	0.06	5.0
2	4.94	0.19	3.9

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	4.4	0.35	8.0
2	1.51	0.07	4.3
3	1.07	0.07	6.6

- **Spiking Recovery**

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

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	1.62	-	-
	2.59	2.62	98.7
	3.52	3.62	97.2
	4.74	4.62	102.6
2	1.92	-	-
	2.81	2.92	96.3
	3.65	3.92	93.1
	5.04	4.92	102.4

- **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	-	3.50	-	-
	2x	1.75	1.75	99.9
	4x	0.95	0.87	108.6
	8x	0.46	0.44	104.1
2	-	3.40	-	-
	2x	1.55	1.70	91.3
	4x	0.93	0.85	109.8
	8x	0.45	0.42	106.0

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

Samples should be stored at –20°C. However, no significant decline in concentration of midkine was observed in serum and plasma samples after 5 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.

Sample	*Incubation Temp., Period*	*Serum (ng/ml)*
1	-20°C	1.89
	2-8°C, 5 days	1.47
	2-8°C, 12 days	1.21
2	-20°C	4.46
	2-8°C, 5 days	4.25
	2-8°C, 12 days	3.80
3	-20°C	3.23
	2-8°C, 5 days	2.84
	2-8°C, 12 days	2.45

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human midkine 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 (ng/ml)*
1	1x	3.34
	3x	3.27
	5x	3.38
2	1x	5.01
	3x	5.19
	5x	4.64
3	1x	1.65
	3x	1.99
	5x	1.89

- **Application for tissue extracts and amniotic fluid**

Human Midkine ELISA kit measures protein isolated from whole tissue homogenates.
Protein extracts isolated from breast and liver tumor tissue showed, in the ELISA, a signal ten times higher as compared to the protein extracts from normal breast and liver tissue.
Recommended dilution for amniotic fluid samples is 150x- 250x.

- **Reference range**

It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for human midkine levels with the assay.

14. DEFINITION OF THE STANDARD

The Standard used in this kit is a recombinant protein with N-terminal fusion of His-tag. It is 14.6 kDa - protein containing 131 amino acid residues (including 10 amino acids of His-tag).

15. METHOD COMPARISON

BioVendor Human Midkine ELISA has not been compared to another 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 Control or samples

17. REFERENCES

References to midkine:

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- Callebaut C. et al.: Inhibition of HIV infection by the cytokine midkine. Virology 281(2): 248-264 (2001)
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- Kosugi T et al.: Growth Factor Midkine Is Involved in the Pathogenesis of Diabetic Nephropathy. Am J Pathol. 168(1): 9–19 (2006)
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- Jia HL et al.: Gene expression profiling reveals potential biomarkers of human hepatocellular carcinoma.Clin Cancer Res. 13(4): 1133-9 (2007)
- Yoshida Y et al.: Expression of the heparin-binding growth factor midkine in the cerebrospinal fluid of patients with neurological disorders. Intern Med. 47(2): 83-9 (2008)
- van der Horst EH et al.: The Growth Factor Midkine Antagonizes VEGF Signaling In Vitro and In Vivo. Neoplasia. 10(4): 340–346 (2008)
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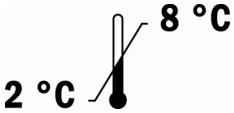
›› References to this product:

- Jia HL et al.: Gene expression profiling reveals potential biomarkers of human hepatocellular carcinoma. Clin Cancer Res. 13(4): 1133-9 (2007)
- Tong Y et al.: Overexpression of midkine contributes to anti-apoptotic effects in human meningiomas. J Neurochem. 100(4):1097-107 (2007)
- Zhang YW et al.: Oligodendrocyte progenitor cells derived from human embryonic stem cells express neurotrophic factors. Stem Cells Dev. 15(6): 943-952 (2006)

›› 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
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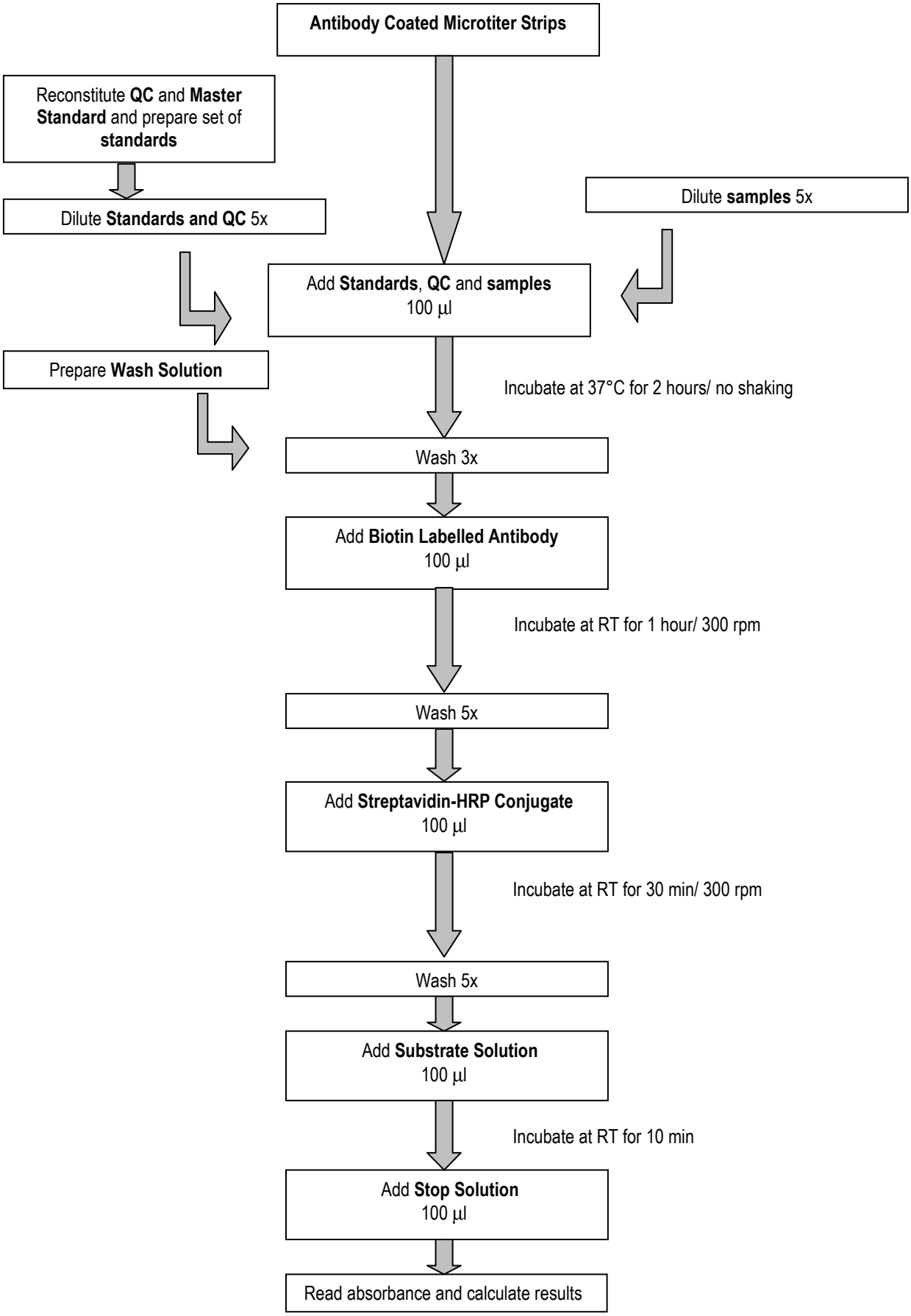
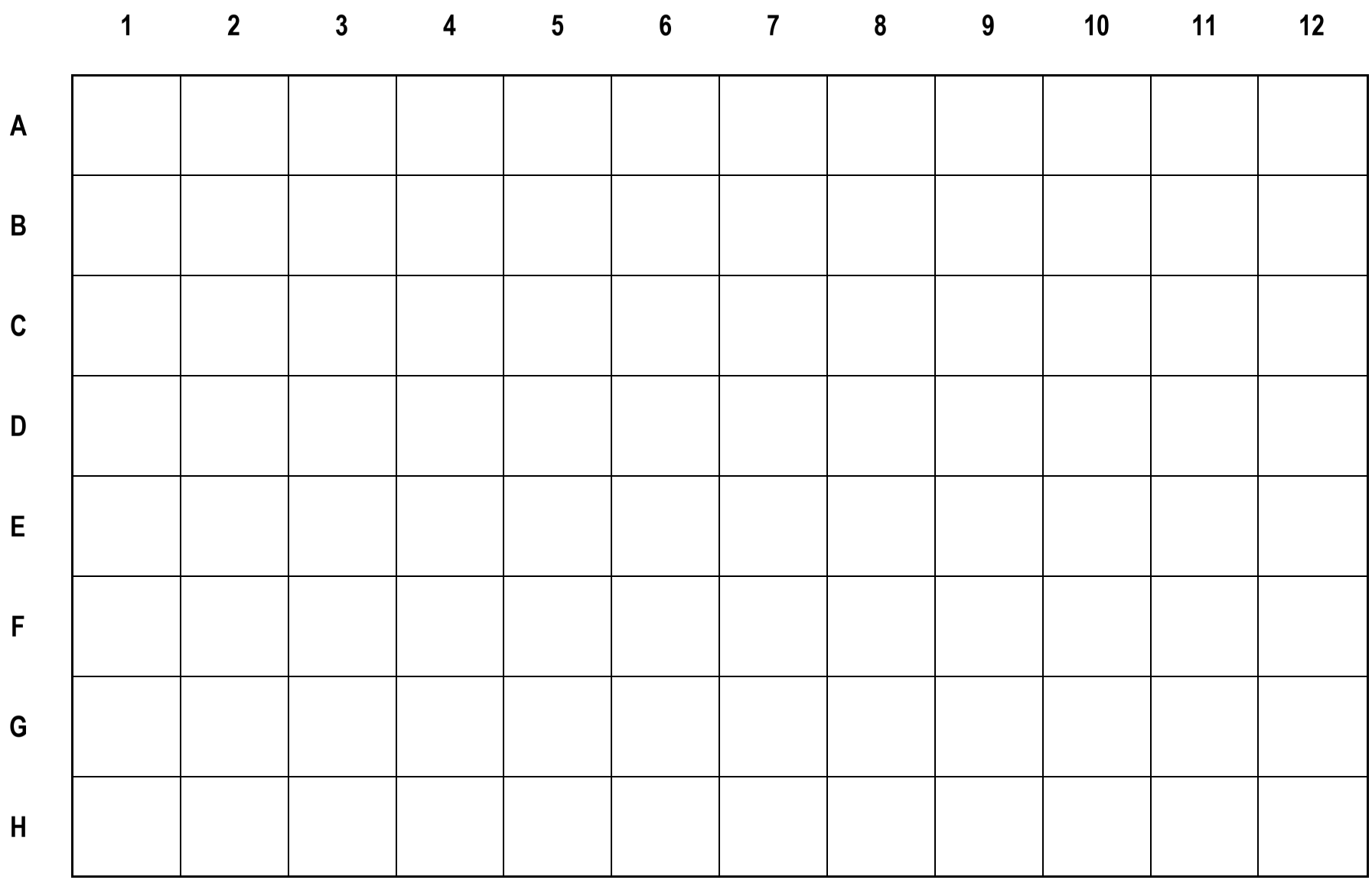
Assay Procedure Summary

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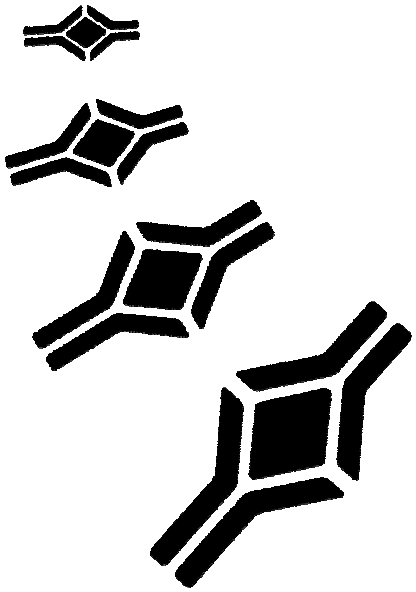

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium
http://www.gentaur-worldwide.com