BioVendor Group

ENG

Instructions for use:

HUMAN HGF ELISA

Catalogue number:

RD194017200R

For research use only!

BioVendor R&D

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HISTORY OF CHANGES

Previous version	Current Version
ENG.001.A	ENG.002.A
”History of changes” added.	
Chapter 9: A sentence „Centrifuge liquid containing microtube vials before opening“ added.	

1. INTENDED USE

The RD194017200R Human HGF ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Hepatocyte Growth factor (HGF).

Features

- **It is intended for research use only**
- The assay time is less than 3.5 hours
- The kit measures HGF in serum 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

Hepatocyte growth factor (HGF) is a multifunctional cytokine that is related to many diseases. HGF is a dimeric molecule, with molecular weight of 84 kDa, composed of an α-subunit 69 kDa and a β-subunit (34 kDa), linked by a disulfide bond.
HGF, also called Scatter factor, is produced by stromal cells, and stimulates epithelial cell proliferation, motility, morphogenesis and angiogenesis in various organs via tyrosine phosphorylation of its receptor, c Met. In fetal stages, HGF-neutralization, or c-Met gene destruction, leads to hypoplasia of many organs, indicating that HGF signals are essential for organ development.
Endogenous HGF is required for self-repair of injured livers, kidneys, lungs and others. In addition, HGF exerts protective effects on organs (including the heart and brain) via anti-apoptotic and anti-inflammatory signals. During organ diseases, blood HGF levels increases significantly. [3]
In animal injury models of the liver, lung, or kidney, the level of HGF activity significantly increased in the damaged organs, with a peak within 1 day after the injury. [3]
In summary, endogenous HGF is important for inducing self-repair responses in numerous organs. The paracrine or endocrine pathway that is predominantly involved in tissue repair depends on the degree, or kind, of injury. [5,8] Regardless of the pathway, HGF is secreted as a pro-HGF and then converted to the active form only at the injury sites by HGF-activators. [6,7] This local activation system may explain the injury-specific c-Met activation that elicits a regenerative response only in an injured organ. [3]
HGF is also involved in cancer invasion and metastasis. The discovery of NK4 as an HGF antagonist has promoted research in cancer biology, pathology and therapy. [4]
Hepatocyte growth factor and its tyrosine kinase receptor (Met) play important roles in myocardial function, both, in physiological and pathological situations. In the developing heart, HGF influences cardiomyocyte proliferation and differentiation. In the adult, HGF/Met signaling controls heart homeostasis and prevents oxidative stress in normal cardiomyocytes.In the injured heart, HGF plays important roles in cardioprotection by promoting: prosurvival (anti-apoptotic and anti-autophagic) effects in cardiomyocytes, angiogenesis, inhibition of fibrosis, anti-inflammatory and immunomodulatory signals, and regeneration through activation of cardiac stem cells. Furthermore, the putative role of elevated HGF as prognostic marker of severity in patients with cardiac diseases is discussed. HGF and HGF based molecules has been examined as new therapeutic tools for the treatment of cardiac diseases. [2]

Areas of investigation:
Cytokines and chemokines and related molecules
Immune Response, Infection and Inflammation
Cardiology
Oncology
Others

4. TEST PRINCIPLE

In the BioVendor Human HGF ELISA, standards and samples are incubated in microplate wells pre-coated with monoclonal anti-human HGF antibody. After 60 minutes incubation and washing, biotin labelled monoclonal anti-human HGF antibody is added and incubated for 60 minutes with captured HGF. 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 HGF. 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 been 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	0.13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
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

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precise 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 microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of shaking at 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.

Centrifuge liquid containing microtube vials before opening.

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 when stored at 2-8°C and protected from the moisture.

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:

Human HGF 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 human HGF in the stock solution is **8000 pg/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	8000 pg/ml
250 µl of stock	250 µl	4000 pg/ml
250 µl of 4000 pg/ml	250 µl	2000 pg/ml
250 µl of 2000 pg/ml	250 µl	1000 pg/ml
250 µl of 1000 pg/ml	250 µl	500 pg/ml
250 µl of 500 pg/ml	250 µl	250 pg/ml
250 µl of 250 pg/ml	250 µl	125 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 Conc. (100x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) to 99 parts Dilution Buffer. Example: 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Dilution Buffer for 1 strip (8 wells).

Stability and storage:
Opened Biotin Labelled Antibody Conc. (100x) is stable 3 months when stored at 2-8°C.
Do not store the 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 HGF in serum and saliva

Samples should 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.

An appropriate dilution should be assessed by the researcher prior to batch measurement.

Recommended starting dilution for serum samples is 3x.
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 100 µl of sample + 200 µl of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Recommended starting dilution for saliva is 5x.

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.

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 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 3-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** 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 paper towel.
7. Pipet **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 3-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 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 HGF 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 8000	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	Standard 4000	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	Standard 2000	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	Standard 1000	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	Standard 500	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	Standard 250	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	Standard 125	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 HGF (pg/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. 500 pg/ml (from standard curve) x 3 (dilution factor) = 1500 pg/ml.

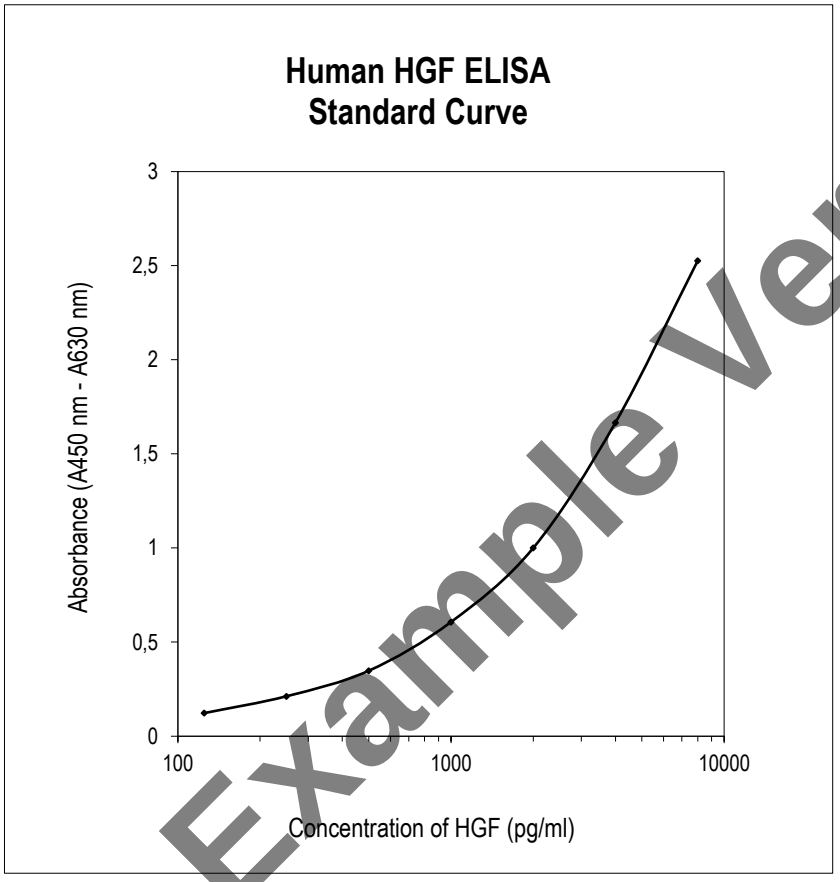

Figure 2: Typical Standard Curve for Human HGF ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human HGF 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 HGF values in wells and is 20 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 (%)
Serum 1	3131	247.7	7.9
Serum 2	5380	206.9	3.9

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

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
Serum 1	1394	67.6	4.9
Serum 2	4083	273.7	6.7

Spiking Recovery

Samples were spiked with different amounts of human HGF and assayed.

Sample	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Serum 1	1072	-	-
	1762	1822	96.7
	2355	2572	91.6
	4117	4072	101.1
Serum 2	1210	-	-
	2020	1960	103.1
	2730	2710	100.7
	3940	4210	93.6

Linearity

Samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Saliva	-	5590	-	-
	2x	2800	2795	102.2
	4x	1300	397	93.0
	8x	600	699	85.9
Serum	-	13038	-	-
	2x	6782	6519	104.0
	4x	3046	3260	93.4
	8x	1531	1630	93.9

14. DEFINITION OF THE STANDARD

WHO Reference Reagent HGF / Scatter Factor, NIBSC code: 96/564 is used as the standard. The standard is CHO-cell derived First International Standard HGF/SF.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 153 unselected donors (87 men + 66 women) 21-65 years old were assayed with the BioVendor Human HGF ELISA in our laboratory.

Age dependent distribution of HGF

Sex	Age (years)	n	HGF (pg/ml)				
			Mean	Median	SD	Min	Max
Men	21-29	17	448	480	135.3	< 375	642
	30-39	25	534	480	220.7	< 375	957
	40-49	31	559	483	193.1	< 375	1059
	50-65	14	580	580	289.1	< 375	1003
Women	22-29	13	463	441	103.2	< 375	657
	30-39	26	541	510	188.1	< 375	891
	40-49	19	617	651	139.3	381	909
	50-61	8	559	522	212.8	< 375	972

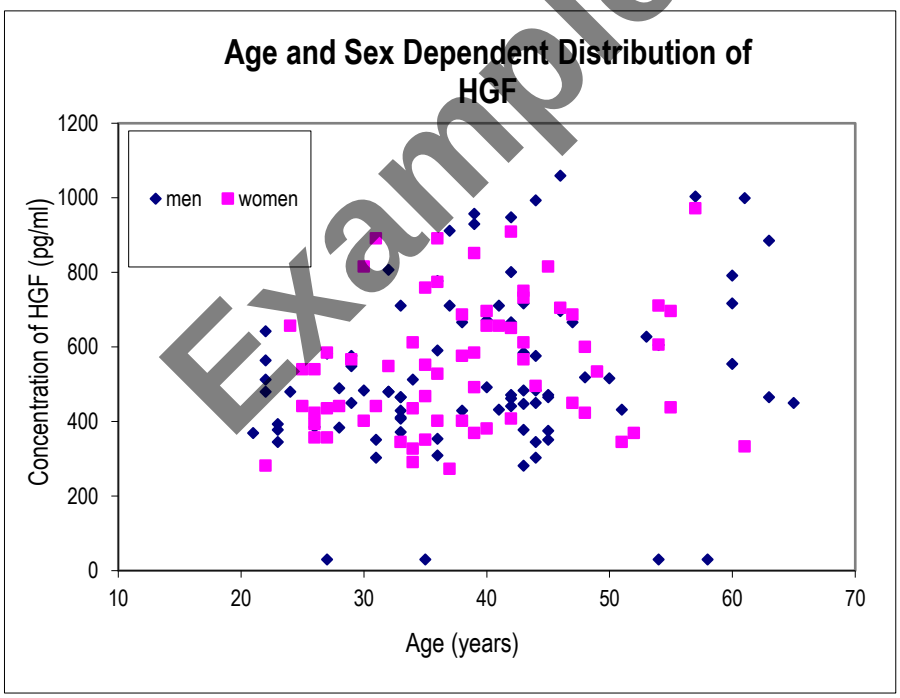

Figure 3: Human HGF concentration plotted against donor age and sex.

Reference range

The data quoted in these instructions should be used for guidance only. 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 references ranges for HGF levels with the assay.

16. METHOD COMPARISON

The BioVendor Human HGF ELISA was compared to another commercial immunoassay by measuring 33 serum samples. The following correlation graph was obtained:

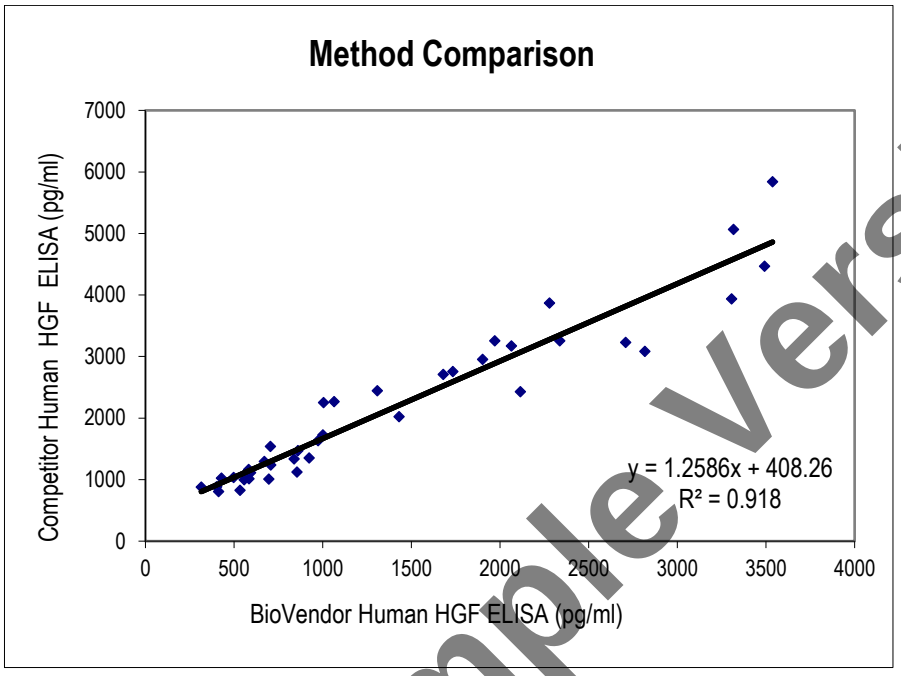

Figure 4: Method Comparison

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 and samples

18. REFERENCES

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4. Matsumoto K, Nakamura T: NK4 gene therapy targeting HGF-Met and angiogenesis. Front. Biosci. 13 (2008)
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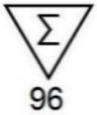
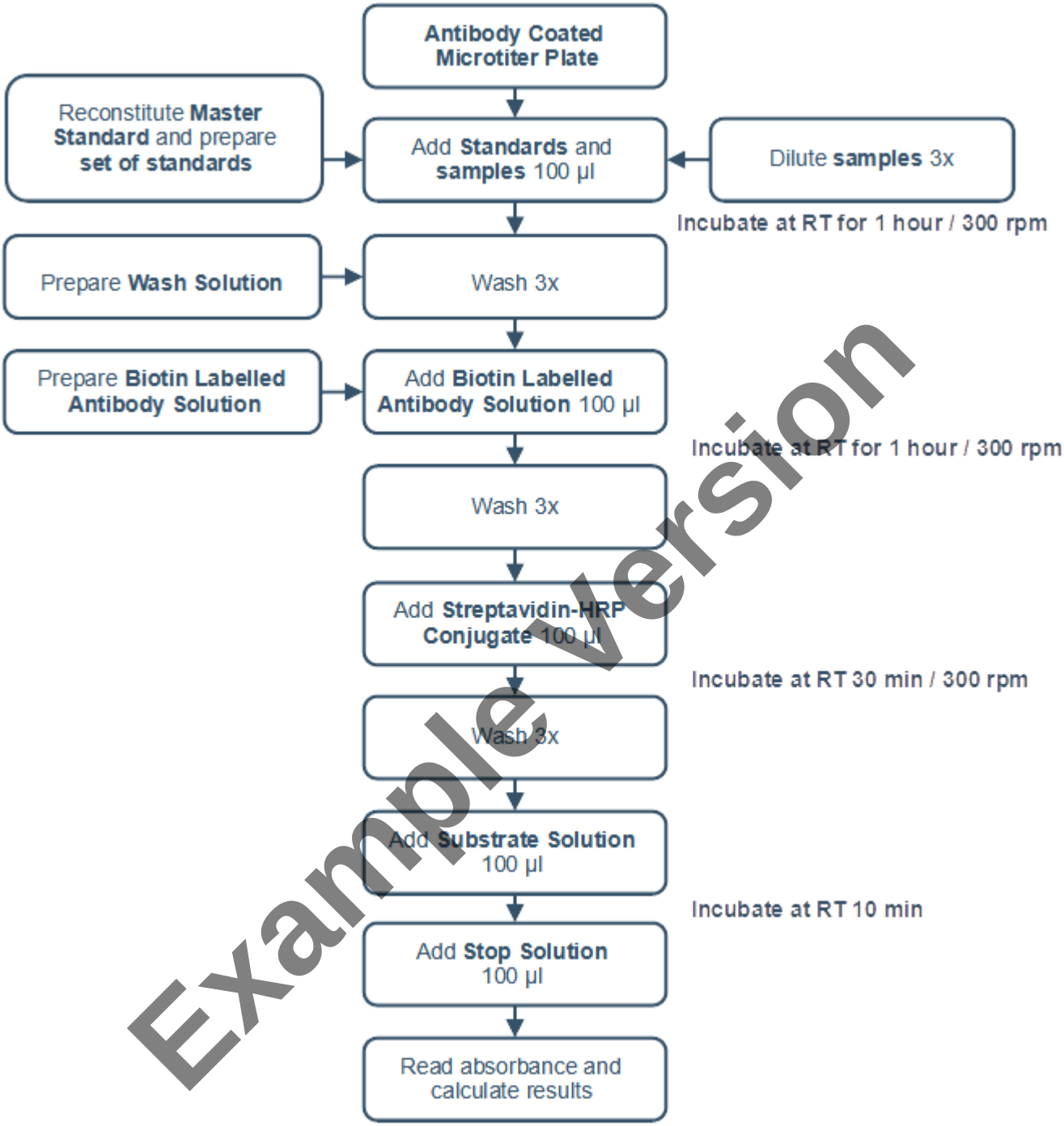
7. Higuchi O, Mizuno K, Vande Woude GF and Nakamura T: Expression of c-met protooncogene in COS cells induces the signal transducing high-affinity receptor for hepatocyte growth factor. FEBS Lett. 301 (1992)
8. Nakamura T: Structure and function of hepatocyte growth factor. Prog. Growth Factor Res. 3 (1991)

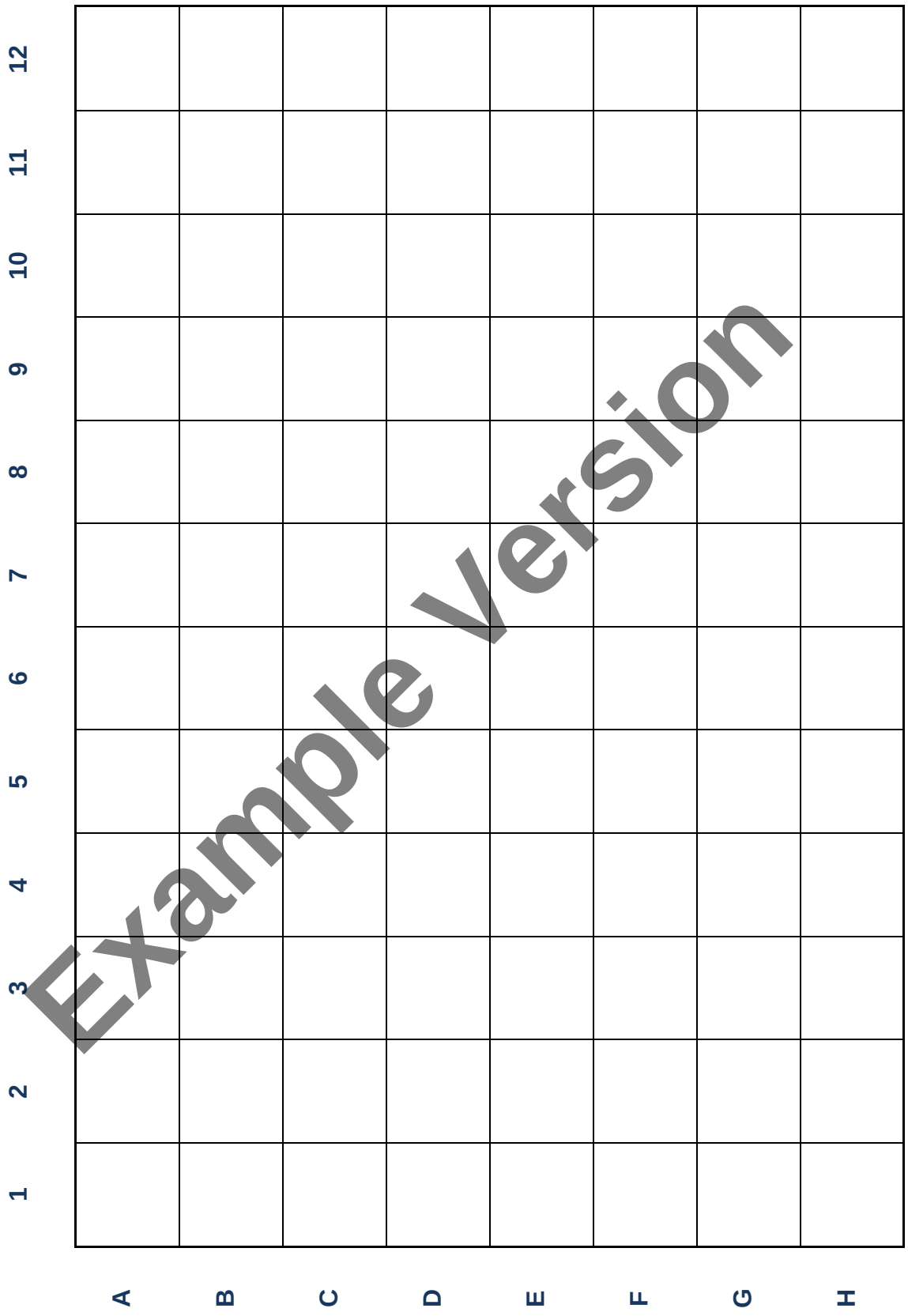
For more references on this product see our web pages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

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

20. ASSAY PROCEDURE - SUMMARY





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