BioVendor Group

ENG

Instructions for Use:

HUMAN MxA PROTEIN ELISA

Catalogue number:

RD194349220R

For research use only!

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.006.A	ENG.007.A
Chapter 9: A sentence “Centrifuge liquid containing microtube vials before opening“ added.	

1. INTENDED USE

The RD194349220R Human MxA Protein ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human MxA protein.

Features

- **It is intended for research use only**
- The total assay time is less than 3 hours
- The kit measures MxA protein in whole blood (cell lysate)
- Assay format is 96 wells
- Standard is recombinant MxA 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

Human MxA protein (Myxovirus resistance protein 1), the product of the MX1 gene, is a 76-kDa protein consisting of 662 amino acid residues and belonging to the dynamic superfamily of large GTPase.
MxA protein plays an important role in intracellular antiviral activity against a wide variety of viruses, including influenza, parainfluenza, measles, coxsackie, hepatitis B virus, and Thogoto virus. The viruses are inhibited by MxA protein at an early stage in their life cycle, soon after host cell entry and before genome amplification. The mouse Mx1 protein (mouse analog of human MxA protein) accumulates in the cell nucleus where it associates with nuclear bodies and inhibits influenza and Thogoto viruses known to replicate in the nucleus. The human MxA protein accumulates in the cytoplasm and endoplasmic reticulum as well. The membrane compartment of endoplasmatic reticulum seems to provide an interaction platform that facilitates viral target recognition. MxA appears to detect viral infection by sensing and trapping nucleocapsid-like structures. As a consequence, the viral components become unavailable for the generation of new virus particles.
The expression of viral MxA protein is induced exclusively and in a dose-dependent manner by IFN-alpha and IFN-beta, but not by IFN-gamma, IL-1, TNF-alpha or other cytokines.
In clinical diagnostics, MxA protein may offer advantages as a marker for viral infection over the other induced proteins such as 2‘, 5‘-oligoadenylate synthetase, because of its very low basal concentration and long half-life. Several clinical studies have reported on the possible use of MxA protein expression in peripheral blood mononuclear cells as a marker for distinguishing viral from bacterial disease, and as a reliable marker for type I IFN bioavailability during IFN treatment of chronic viral hepatitis and multiple sclerosis. Myxovirus resistance protein A (MxA) can be used as a marker of the bioactivity of interferon-beta (IFN-beta) therapy for patients with multiple sclerosis (MS). Two to forty per cent of IFN-beta-treated multiple sclerosis (MS) patients develop IFN-beta-neutralizing antibodies (NAb) with subsequent attenuation of MxA protein induction. The MxA ELISA could be used for assessment of raised NAb.

Areas of investigation:
Viral Infection
Multiple Sclerosis

4. TEST PRINCIPLE

In the BioVendor Human MxA Protein ELISA, Standards and samples are incubated in microtitration wells pre-coated with monoclonal anti-human MxA protein antibody. After 60 minutes incubation followed by washing, biotin labelled monoclonal anti-human MxA protein antibody is added and incubated with the captured MxA protein 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 MxA protein. A standard curve is constructed by plotting absorbance values against MxA protein concentrations of Standards and concentrations of unknown samples are determined using this standard curve.

Since human MxA protein is found mainly in mononuclear cells, lysis of blood cells is required before running the assay.

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 Concentrate (100x)	concentrated	0.13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Buffer for cell lysis	ready to use	15 ml
Dilution Buffer	ready to use	2 x 13 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 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.

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 desicant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8 °C and protected from the moisture.

Streptavidin-HRP Conjugate

Buffer for cell lysis

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 MxA Protein 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 occasionally gently shaking (not to foam). The resulting concentration of the MxA protein in the stock solution is **6 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	6 ng/ml
250 µl of 6 ng/ml	250 µl	3 ng/ml
250 µl of 3 ng/ml	250 µl	1.5 ng/ml
250 µl of 1.5 ng/ml	250 µl	0.75 ng/ml
250 µl of 0.75 ng/ml	250 µl	0.375 ng/ml
250 µl of 0.375 ng/ml	250 µl	0.188 ng/ml

Prepared Standards are ready to use, do not dilute them.

Stability and storage:
Do not store the reconstituted 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) with 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:
Do not store the diluted Biotin Labelled Antibody solution.

Wash Solution Concentrate (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 MxA protein in whole blood (cell lysate).

Whole blood samples should be collected in EDTA collection tubes and assayed preferably immediately after collection or could be stored at -20 °C (-80 °C).
Cell lysis: Dilute whole blood samples just prior to the assay 10x with Buffer for cell lysis, e.g. 15 µl of sample + 135 µl of Buffer for cell lysis. Mix well (not to foam) and incubate for 30 minutes at room temperature.
Dilute lysed samples 2x with Dilution Buffer e.g. 150 µl of lysed sample + 150 µl of Dilution Buffer. Mix well (not to foam). Vortex is recommended.

Stability and storage:
Whole blood samples are stable at 4 °C for 24 hours. Whole blood samples should be stored at -20 °C, or preferably at -80 °C, for long-term storage.

See Chapter 13 for effect of freezing/thawing of whole blood sample on the concentration of human MxA.

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.
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. Add **100 µl** of Biotin Labelled Anti-MxA 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. 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).
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 **20 minutes** at room temperature. The incubation time may be extended [up to 30 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: 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 MxA protein 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 6**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	**Standard 3**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	**Standard 1.5**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	**Standard 0.75**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	**Standard 0.375**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	**Standard 0.188**	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 MxA protein (ng/ml) in samples.

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. 1.5 ng/ml (from standard curve) x 20 (dilution factor) = 30 ng/ml.

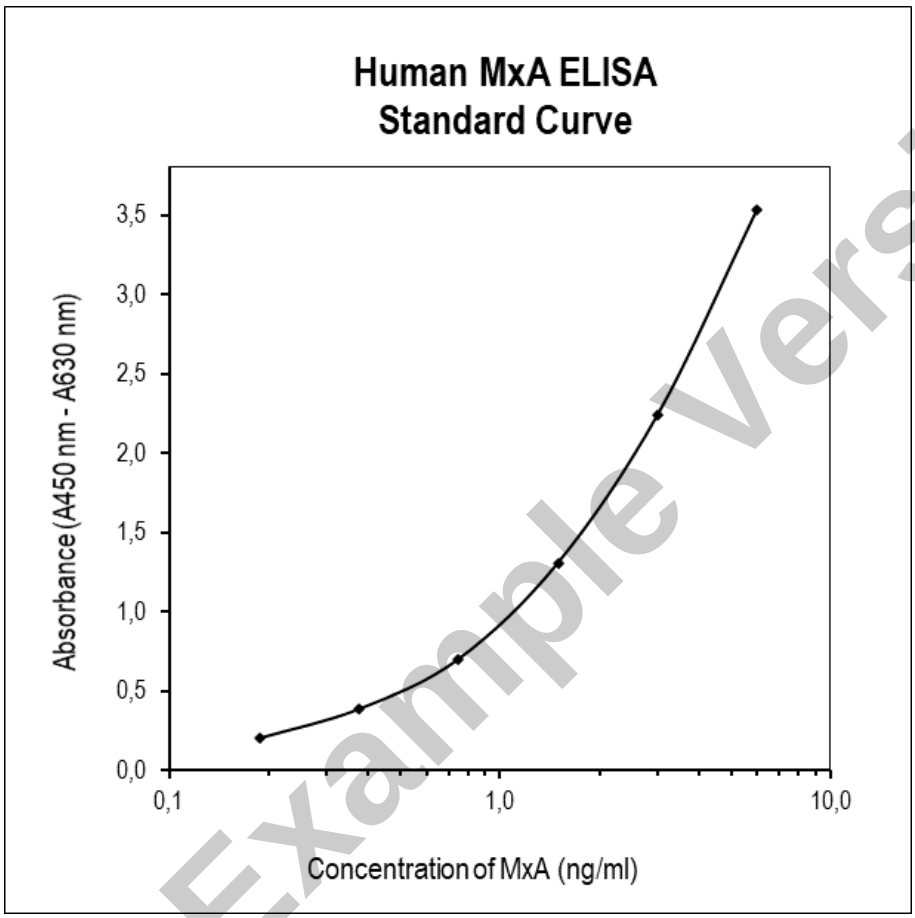


Figure 2: Typical Standard Curve for Human MxA Protein ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human MxA Protein 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 human MxA in wells and is 0.008 ng/ml.
*Dilution Buffer is pipetted into blank wells.

Limit of assay

Results exceeding resistin MxA protein of 6 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the MxA protein concentration.

Interference Study

The antibodies used in this ELISA are specific for human MxA.
We observed no interference of hemoglobin (2.0 mg/ml), bilirubin conjugated (0.2 mg/ml), bilirubin unconjugated (0.2 mg/ml), triglycerides (20 mg/ml) and biotin (3500 ng/ml).

For details please contact us at info@biovendor.com.

Presented results are multiplied by respective dilution factor

Precision

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

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	21.37	1.28	6.0
2	36.82	1.81	4.9

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

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	14.97	1.03	6.9
2	51.41	2.07	4.0

Spiking Recovery

Blood samples were spiked with different amounts of human MxA protein and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	11.56	-	-
	17.32	19.06	90.9
	22.26	26.56	83.8
	35.12	41.56	84.5
2	3.26	-	-
	6.74	7.02	96.0
	10.54	10.76	98.0
	15.76	18.26	86.3

Linearity

Blood samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	-	26.34	-	-
	2x	14.10	13.17	107.1
	4x	7.24	6.59	109.9
	8x	3.42	3.29	103.9
2	-	47.38	-	-
	2x	20.14	23.69	85.0
	4x	9.70	11.85	81.9
	8x	4.74	5.92	80.0

Effect of Freezing/Thawing

No decline was observed in concentration of human MxA in whole blood samples stored at -20 °C and -80 °C 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	Samples at -20 °C (ng/ml)	Samples at -80 °C (ng/ml)
1	1x	18.77	18.63
	3x	22.33	20.69
	5x	20.41	16.60
2	1x	42.24	38.43
	3x	45.35	38.87
	5x	44.25	35.18
3	1x	57.09	52.94
	3x	59.86	57.54
	5x	48.53	55.95

Reference range

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 MxA levels with the assay.

14. METHOD COMPARISON

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

15. 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

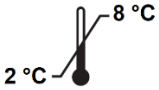
16. REFERENCES

References to MxA protein:

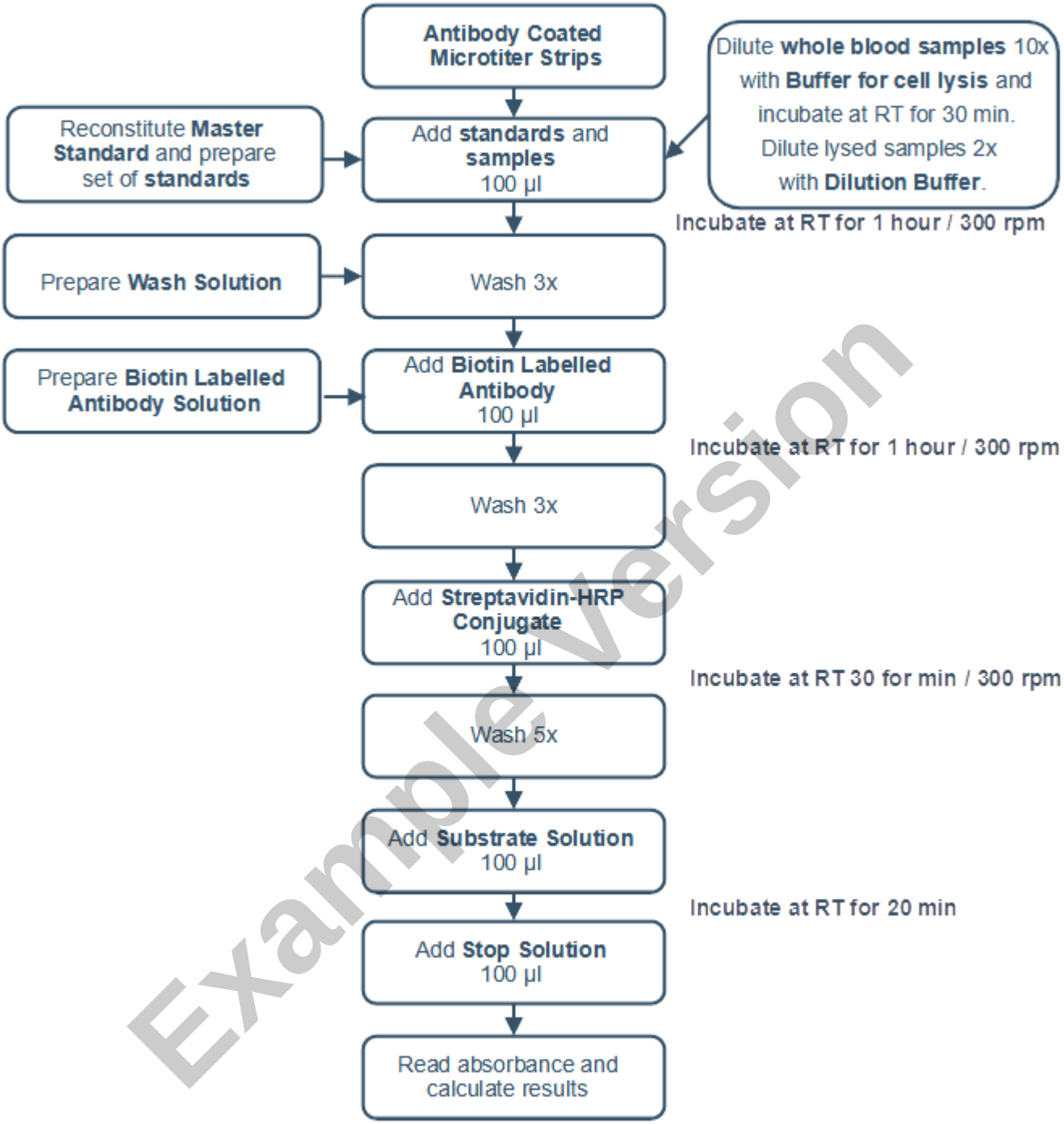
- Bachur et al., Predictive model for serious bacterial infections among infants younger than 3 months of age. Pediatrics, 108, 311-316 (2001)
- Haller et al., Interferon-induced mx proteins: dynamin-like GTPases with antiviral activity. Traffic, 3, 710-717 (2002)
- Halminen et al., Expression of MxA protein in blood lymphocytes discriminates between viral and bacterial infections in febrile children. Pediatr. Res., 41, 647-650 (1997)
- Huppert et al., Clinical value of measuring the interferon induced enzyme 2', 5'-oligoadenylate synthetase in children. Acta Paediatr., 81, 329-334 (1992)
- Chieux et al., The MxA protein levels in whole blood lysates of patients with various viral infection. J. Virol. Methods, 70, 183-191 (1998)
- Kawamura et al., New sandwich-type enzyme-linked immunosorbent assay for human MxA protein in a whole blood using monoclonal antibodies against GTP-binding domain for recognition of viral infection. J Clin Lab Anal., 26 (3), 174-83 (2012)
- Koskenvuo et al., Expression of MxA protein in blood lymphocytes of children receiving anticancer chemotherapy. Pediatric Hematology and Oncology, 23, 649-660 (2006)
- Nakabayashi et al., MxA-based recognition of viral illness in febrile children by a whole blood assay. Pediatr.Res., 60, 770-774 (2006)
- Simon et al., Interferon-regulated Mx gene are not responsive to IL-1, TNF and other cytokins. J. Virol., 65, 968-971 (1991)
- Vallittu et al., MxA protein assay for optimal monitoring of IFN-beta bioactivity in the treatment of MS patients. Acta. Neurol. Scand., 118, 12-17 (2008)

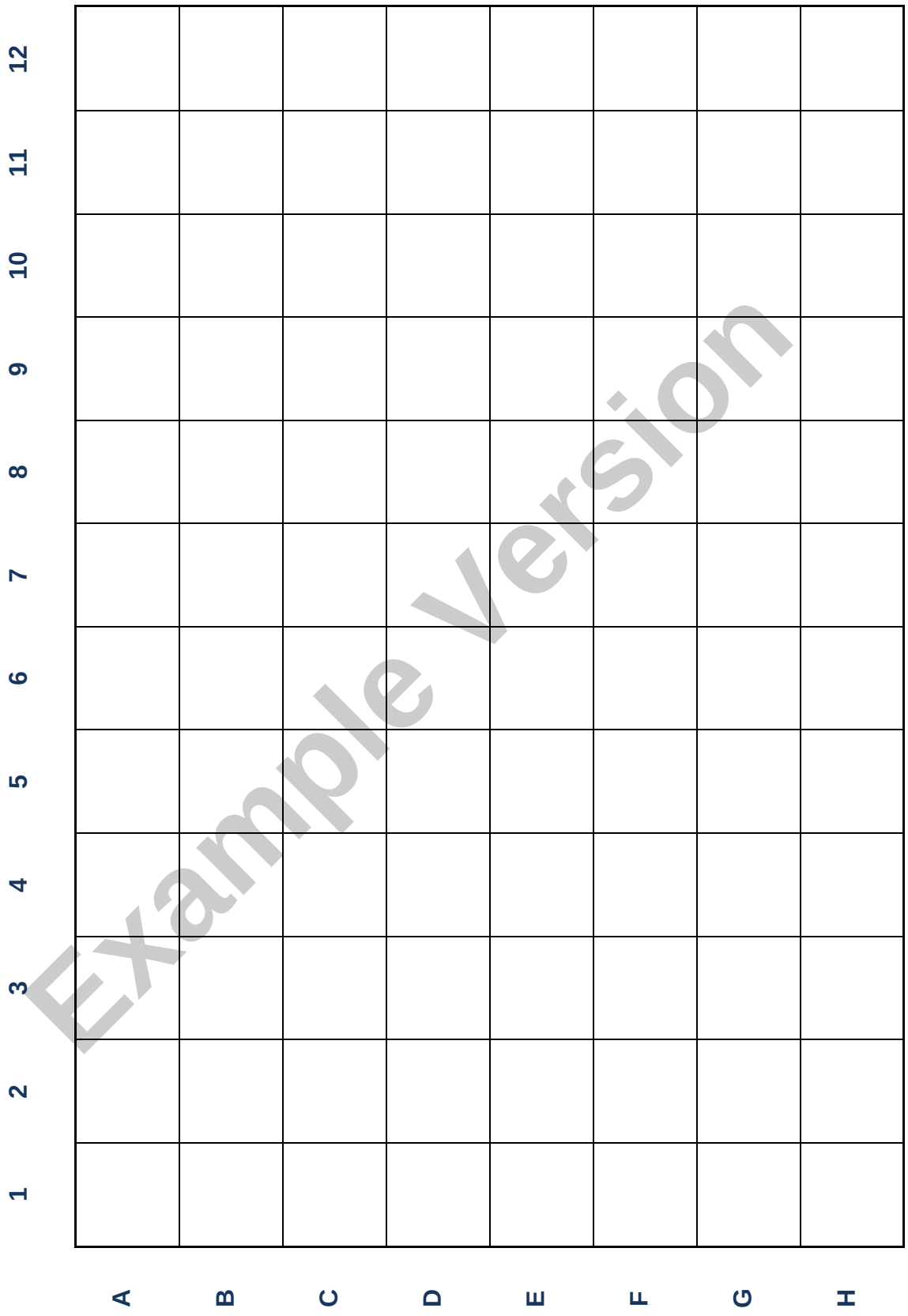
For more references on this product see our web pages at www.biovendor.com.

17. 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

18. ASSAY PROCEDURE - SUMMARY





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