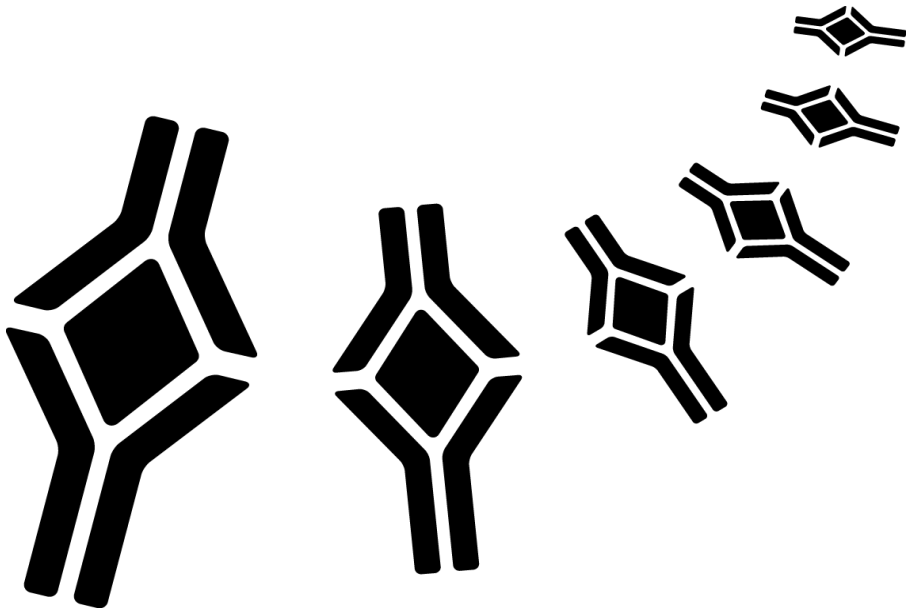
BioVendor
Research
and Diagnostic Products



HUMAN MATRIX METALLOPROTEINASE-3 ELISA

Product Data Sheet

Cat. No.: RD191510100CS

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 RD191510100CS Human Matrix Metalloproteinase-3 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human matrix metalloproteinase-3 (MMP 3).

Features

- **It is intended for research use only**
- The total assay time is less than 3 hours
- The kit measures human matrix metalloproteinase-3 in human 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

Matrix metalloproteinases (MMPs) are a group of enzymes engaged in the degradation and remodeling of extracellular matrix (ECM). Nowadays six groups of these enzymes have been distinguished (collagenases, gelatinases, stromelysins, matrilysins, membrane-type, and a sixth group encompassing several other MMPs not classified in the previous categories), differing in structure, cellular localization, and substrate specificity. Since these enzymes are involved in connective tissue remodeling occurring in the course of morphogenetic processes, therefore, they are a subject of a very strict regulation, which is executed, among others, by the expression of their specific inhibitors—tissue inhibitors of metalloproteinases (TIMPs).
MMP-3 (also referred to as stromelysin-1) may be expressed in fibroblasts, chondrocytes, endothelial cells, macrophages, vascular smooth muscle cells, osteoblasts, and keratinocytes in response to appropriate stimuli. Various agents regulate its biosynthesis. Inflammatory cytokines such as IL-1 and TNF-α, epidermal growth factor, platelet-derived growth factor, phorbol and oncogenic cellular transformation are the inductive agents. In comparison, retinoic acid, glucocorticoids, estrogen, progesterone and TGF-β suppress MMP-3 synthesis.
MMP-3 is secreted from the cells as a proenzyme. The proenzyme has been shown to stimulate plasminogen activation. The N-terminal pro-domain contains the cysteine switch motif conserved in MMPs that maintains MMP-3 in the latent stat. Activation of the proenzyme results in the removal of the pro-domain. MMP-3 activation can be achieved in vitro by proteases such as itself, chyrotrypsin, neutrophil elastase and plasma kallikrein, and by mercury compounds. The resulting active enzyme consists of a catalytic domain with a zinc-binding motif conserved in metzincins. A short hinge peptide links the catalytic domain to the C-terminal hemopexin-like domain.MMP-3 hydrolyzes components of the extracellular matrix like proteoglycan, laminin, fibronectin, gelatin and collagen types III, IV and IX. It also activates pro-MMP-9 and pro-MMP-8 and superactivates plasmin activated MMP-1. MMP-3 is secreted as a latent proenzyme and is activated by a variety of proteinases, e.g. plasmin, trypsin, chymotrypsin, cathepsin G or human neutrophil elastase. MMP-3 was found to be capable of activating the precursor of IL1-beta.

Areas of investigation:
Rheumatology
Inflammatory diseases
Cardiovascular disorders
Cancer

4. TEST PRINCIPLE

In the BioVendor Human Matrix Metalloproteinase 3 ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human MMP-3 antibody. After 60 minutes incubation and washing, polyclonal anti-human MMP-3 antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured MMP-3. Following another 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 spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of MMP-3. 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 mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENTS SUPPLIED

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution	ready to use	13 ml
Master Standard	lyophilized	2 vials
Dilution Buffer	ready to use	2x13 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 (Dilution Buffer)
- Precision pipettes to deliver 10 – 1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Plate cover
- 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 - 8 °C and protected from the moisture.

Conjugate Solution
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 MMP-3 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 MMP-3 in the stock solution is **10 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	10 ng/ml
250 µl of stock	250 µl	5 ng/ml
250 µl of 5 ng/ml	375 µl	2 ng/ml
250 µl of 2 ng/ml	250 µl	1 ng/ml
250 µl of 1 ng/ml	250 µl	0.5 ng/ml
250 µl of 0.5ng/ml	375 µl	0.2 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.

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 MMP-3 in serum and saliva.

Samples can be assayed immediately after collection, or after long-term storage. 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 in advance to batch measurement.

Recommended starting dilution for serum nad plasma is 15x.
Dilute samples (serum, plasma) 15x with Dilution Buffer just prior to the assay, e.g. 10 µl of sample + 140 µl of Dilution Buffer for singlets or 20 µl of sample + 280 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

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

See Chapter 13 for effect of sample matrix (serum/plasma) on the concentration of human matrix metalloproteinase 3.

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 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. Add **100 µ** Conjugate 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 Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for **15 minutes** at room temperature. The incubation time may be extended if the reaction temperature is below than 20 °C. Do not shake the plate during the incubation.
9. Stop the colour development by adding **100 µl** of Stop Solution.
10. 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 10.**

Note: If some samples and standard/s have absorbance 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 matrix metalloproteinase-9 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 5-times: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat four 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 10.0**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 5.0**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 2.0**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 1.0**	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	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microtiter plate readers perform automatic calculations of analyte concentration. The Standards curve is constructed by plotting the mean absorbance of Standards (Y) against the known concentration of Standards (X) in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of MMP-3 (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 of Standards (X).

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. 4 ng/ml (from standard curve) x 15 (dilution factor) = 60 ng/ml.

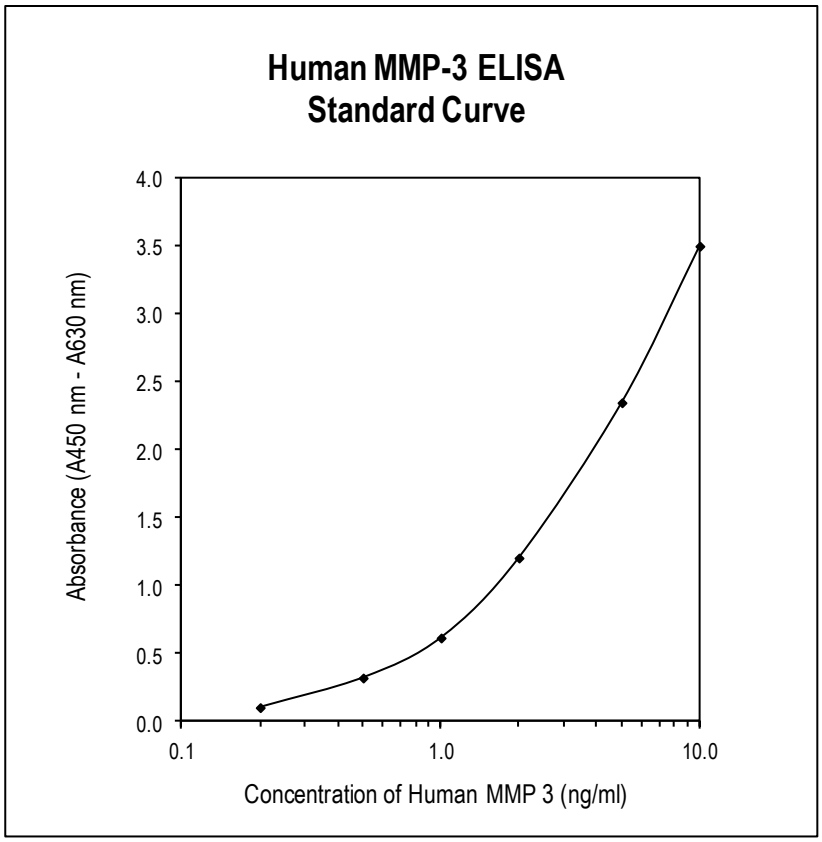


Figure 2: Typical standard curve for Human MMP-3 ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human MMP-3 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 MMP-3 values in wells and is: 0,1 ng/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.

- **Specificity**

The antibodies used in this ELISA are specific to human matrix metalloproteinase-3 with no significant crossreactivities to recombinant matrix metalloproteinase-8, matrix metalloproteinase-9 or TIMP-1 (all at 20 ng/ml) in Human Matrix Metalloproteinase-3 ELISA.

» Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	43.53	1.6	3.7
2	15.32	1.483	5.5

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	55.73	5.3	9.4
2	16.01	1.5	8.46

- **Spiking Recovery**

Serum samples were diluted, spiked with different amounts of human MMP-3 and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
Serum 1	8.49	-	-
	11.55	12.24	94.4
	15.52	15.99	97.1
	22.62	23.49	96.3
Serum 2	8.62	-	-
	11.61	12.37	93.9
	15.70	16.12	97.4
	22.60	23.62	95.7

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
Serum 1	-	46.33	-	-
	2x	21.66	23.17	93.5
	4x	10.80	11.58	93.2
	8x	5.82	5.79	100.5
Serum 2	-	16.53	-	-
	2x	7.72	8.27	93.5
	4x	3.96	4.13	95.8
	8x	2.37	2.07	114.7

The recombinant human matrix metalloproteinase-3 (MMP-3) is used as the standard. The recombinant matrix metalloproteinase-3 (MMP-3) expressed in HEK293 is a 53 kDa protein consisting of 466 amino acid residues of human matrix metalloproteinase-3 (MMP-3).

14. PRELIMINARY POPULATION DATA

The following results were obtained when serum samples from 80 unselected donors (37 female + 43 male, with minimum age of 28, maximum age of 83 and mean age of 60.58) were assayed with the Biovendor Human MMP-3 ELISA in our laboratory.

n	MMP-3				
	Mean	Median	SD	Min	Max
80	17.91	14.70	13.66	1.20	110.90

- **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 matrix metalloproteinase-3 (MMP-3) levels with the assay.

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:

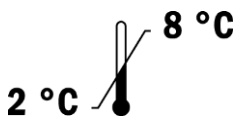
- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30 °C

›› High coefficient of variation (CV)

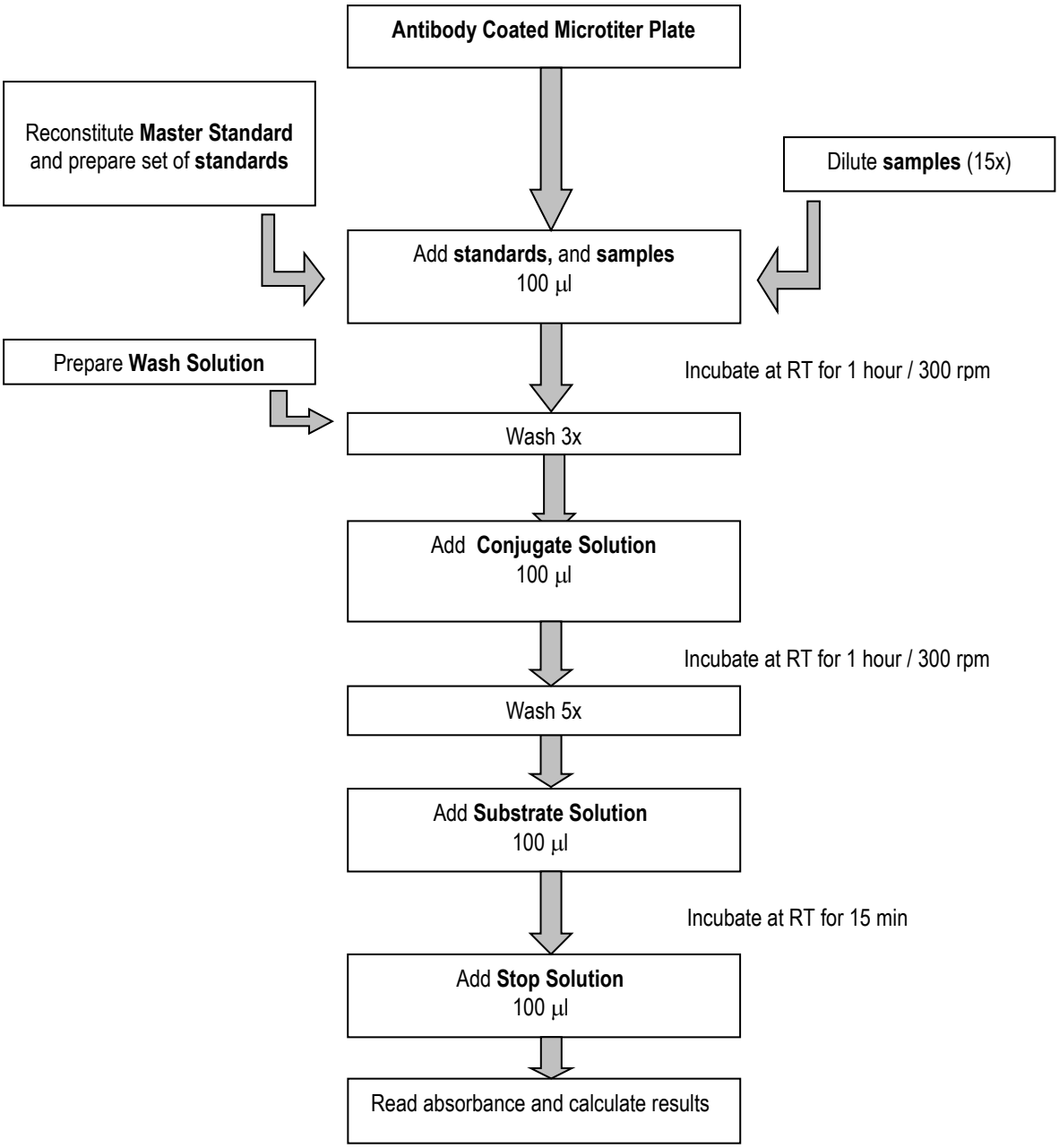
Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards or samples

16. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	Attention, see instructions for use
	Potential biological hazard
	Expiry date
	Storage conditions
	Name and registered office of the manufacturer

Assay Procedure Summary



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B												
C												
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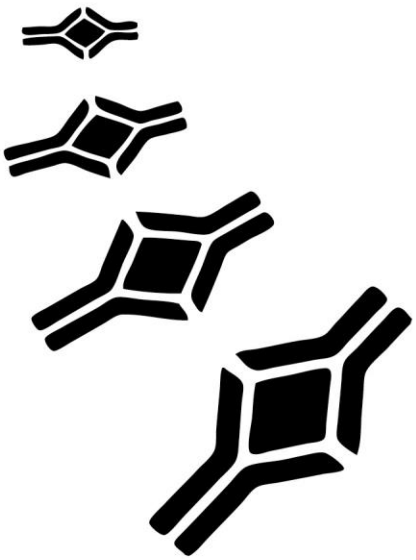

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