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Research
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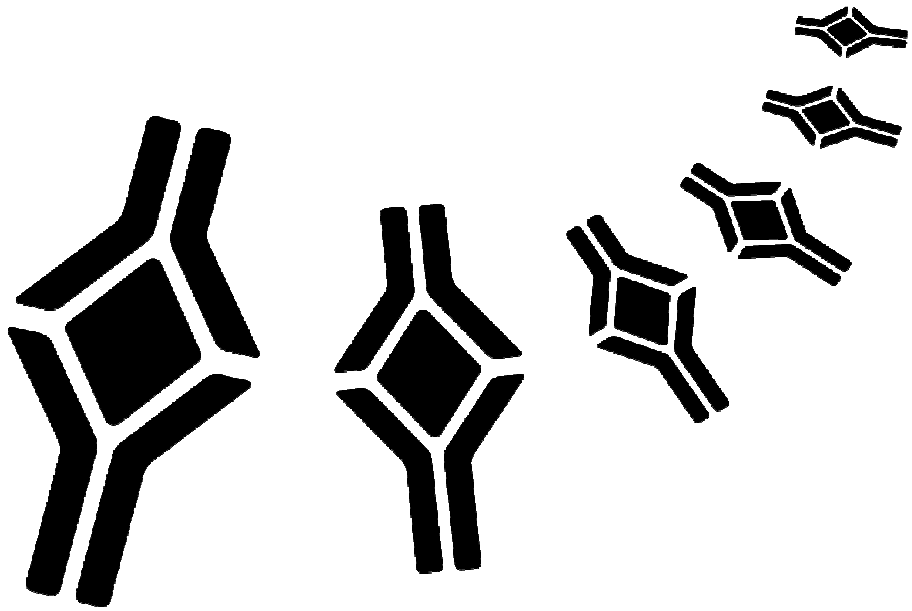

HUMAN CYSTATIN B ELISA

Product Data Sheet

Cat. No.: RD193398200CS

For research use only!

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This kit is manufactured by:
BioVendor – Laboratorní medicína a.s.

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Use only the current version of Product Data Sheet enclosed with the kit!

1. INTENDED USE

The RD193398200CS Human Cystatin B ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human cystatin B (CSTB).

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

Cystatin B (CSTB), also known as stefin B, is an endogenous inhibitor of cysteine proteinases localized in the nukleus and the cytosol.[1]
Stefins are 11 kDa intracellular proteins, which are ubiquitously expressed in human tissues. They belong to the family of cystatins, which are endogenous cysteine protease inhibitors specific for the papain-family of cysteine proteinases and are classified as the IH clan in the MEROPS scheme. Stefin B is a globular protein of 98 amino acid residues which does not contain disulphide bonds (it has one cysteine at site 3) or carbohydrate groups.[2]
Cystatin B belongs to the cystatins superfamily which has primarily been explored with respect to its capacity to inhibit intracellular cysteine proteases leaking from lysosomes and has been implicated in several types of cancers, such as breast, lung and colorectal cancers, glio-blastoma, squamous cell carcinoma of the head and neck, laryngeal, esophageal and hepatocellular carcinomas, and prostatic adenocarcinoma. The dysregulated expression of CSTB appears to be associated with tumorigenesis and may be mediated by a variety of cytokines and growth factors, including transforming growth factor-β (TGF-β).[3]
In the nervous system, Cystatin B is found in neural stem cells, neurons, glia, astrocytes and neuroepithelium. Cystatin B can also interact with amyloid-β, and Cystatin B tetramers are specifically found to inhibit amyloid-β fibril growth.
In rheumatoid arthritis, serum Cystatin B is reported to correlate with joint destruction and swelling.

<u>Clinical use and areas of investigation:</u>
Autoimmunity
Cancer

4. TEST PRINCIPLE

In the BioVendor Human Cystatin B ELISA, standards and samples are incubated in microplate wells pre-coated with anti-human cystatin B antibody. After 60 minutes incubation and washing, biotin-labelled polyclonal anti-human cystatin B antibody is added and incubated with captured cystatin B for 60 minutes. After another washing, streptavidin-horseradish peroxidase 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 cystatin B. 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 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
Dilution Buffer	ready to use	20 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 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 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 Labelled Antibody
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 Cystatin B 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 cystatin B in the stock solution is **2.5 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	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
250 µl of 0.1 ng/ml	250 µl	0.05 ng/ml

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

Stability and storage:
Standard stock solution (2.5 ng/ml) should be aliquoted and frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.
Do not store the 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 cystatin B in serum and urine.

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 or urine samples 15x with Dilution Buffer just prior to the assay, e.g. 20 µl of sample + 280 µl of Dilution Buffer for both singlets, and 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.

Ask for information at info@biovendor.com if assaying other matrices.

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 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 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 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). 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: 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 cystatin B 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 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 2.5**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 1**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 0.5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 0.25**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	**Standard 0.1**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	**Standard 0.05**	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 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 cystatin B 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. 0.5 ng/ml (from standard curve) x 15 = 7.5 ng/ml.

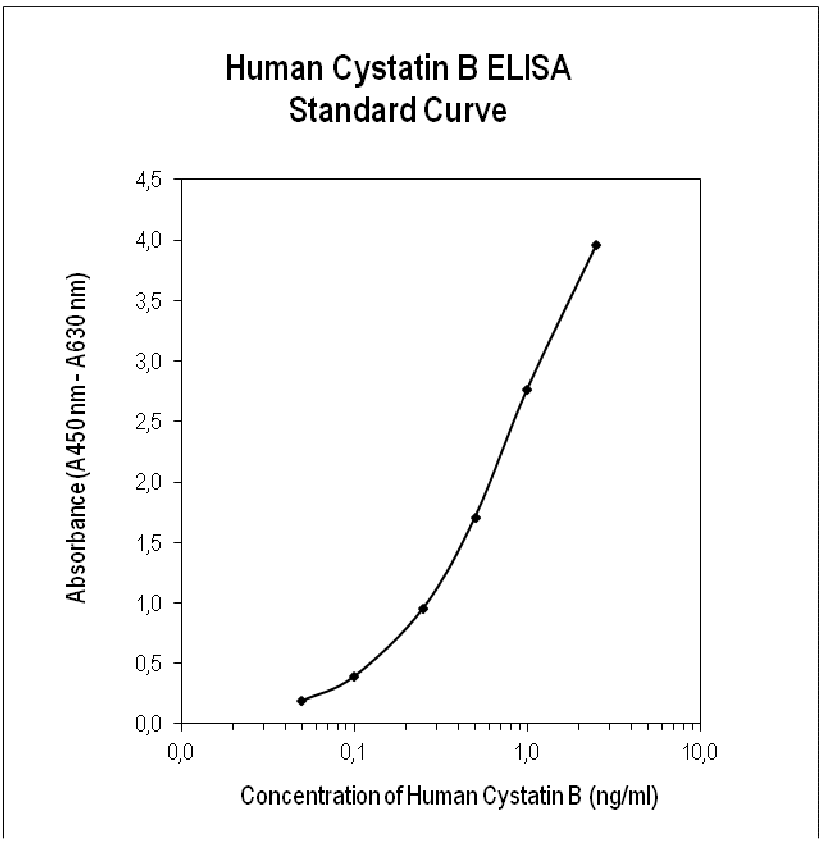


Figure 2: Typical Standard Curve for Human Cystatin B ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human Cystatin B ELISA are presented in this chapter

- **Sensitivity**

Pending data.

- **Limit of assay**

Pending data

- **Specificity**

Pending data.

- **Precision**

Pending data.

- **Spiking Recovery**

Pending data.

- **Linearity**

Pending data.

14. DEFINITION OF THE STANDARD

The recombinant protein produced in *E. coli*-derived cell line is used as the Master Standard in this assay. The cystatin B is a 12 kDa protein consisting of 96 amino acids, with N-terminal Met and 7-His tag.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 80 unselected donors were assayed with the Biovendor Human Cystatin B ELISA in our laboratory.

n	Cystatin B				
	Mean	Median	SD	Min	Max
80	4.38	3.16	2.15	0	35.32

The following results were obtained when urine samples from 222 donors (60 female + 162 male, with minimum age of 27, maximum age of 94 and mean age of 70.23) were assayed with the Biovendor Human Cystatin B ELISA in our laboratory:

Age and Sex dependent distribution of human Cystatin B

Age (years)	n	Cystatin B (ng/ml)				
		Mean	Median	SD	Min	Max
27-59	27	2.57	1.57	3.10	0.10	14.64
60-69	73	3.44	2.11	3.62	0.17	15.58
70-79	78	3.63	1.88	4.67	0.02	24.58
80-94	44	4.17	2.36	4.24	0.24	19.39

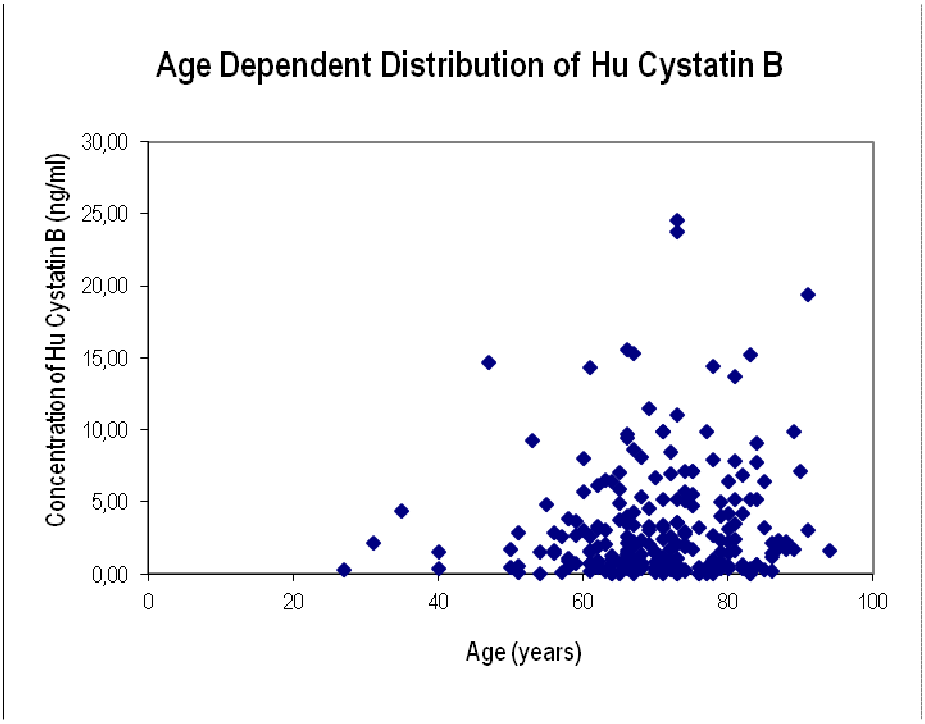


Figure 4: Human Cystatin B concentration plotted against donor age.

16. REFERENCES

References to Cystatin B:

1 Sun T. Increased expression of stefin B in the nucleus of T98G astrocytoma cells delays caspase activation. Front Mol Neurosci. 2012 Sep 14;5:93

2 Taler-Verčič A. The role of initial oligomers in amyloid fibril formation by human stefin B. Int J Mol Sci. 2013;14(9):18362-84

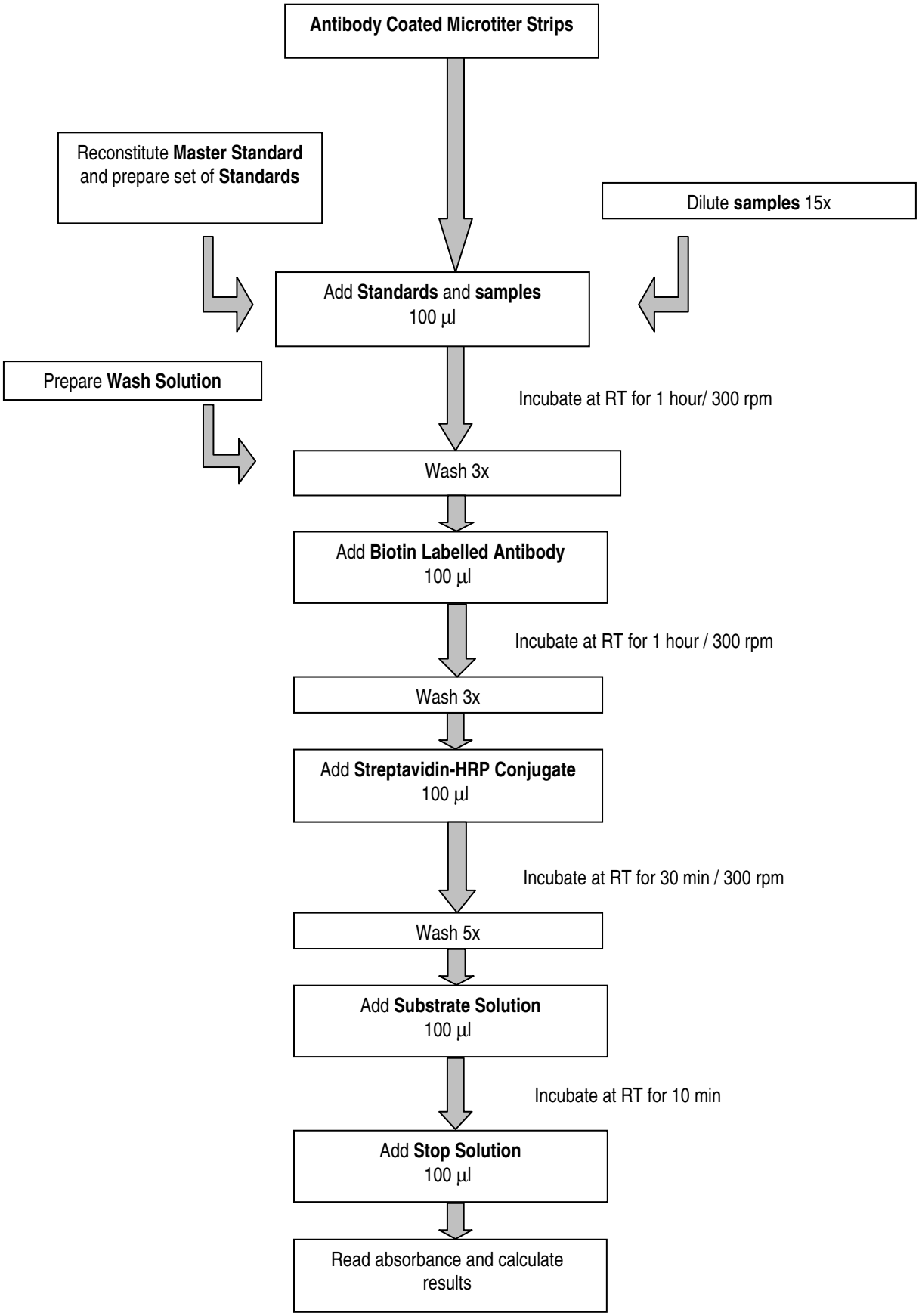
3 Wang X. Cystatin B is a progression marker of human epithelial ovarian tumors mediated by the TGF-β signaling pathway. Int J Oncol. 2014;44(4):1099-106

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

17. 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
IVD CE	In vitro diagnostic medical device

Assay Procedure Summary



	1	2	3	4	5	6	7	8	9	10	11	12
A												
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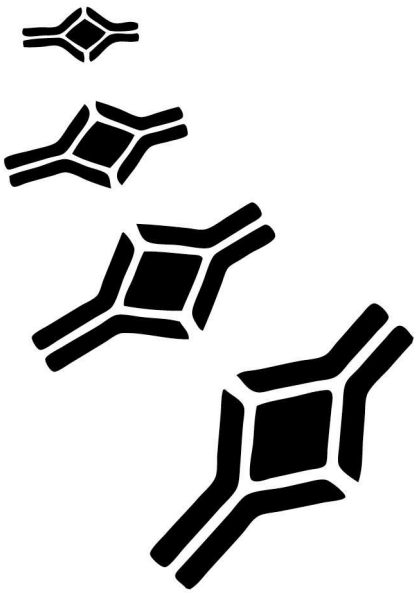

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