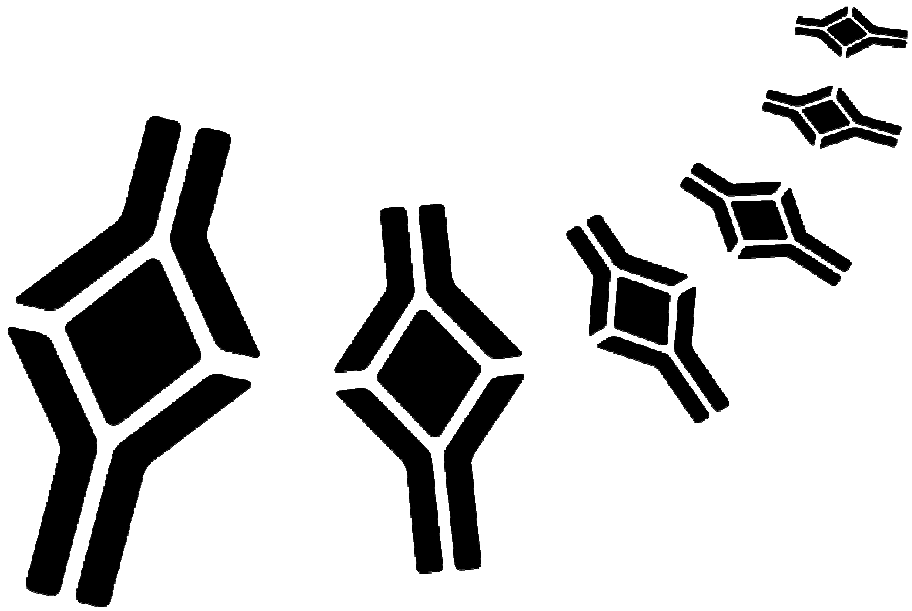
BioVendor
Research
and Diagnostic Products



HUMAN CHITINASE 3-LIKE 1 ELISA

Product Data Sheet

Cat. No.: RD193444200CS

For research use only!

CONTENTS

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

Development of the product was supported by Ministry of Industry and Trade of the Czech Republic, project No. FR-TI3/666.

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

1. INTENDED USE

The RD193444200CS Human Chitinase 3-Like 1 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human chitinase 3-like 1 (CHI3L1).

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

Chitinase 3-like 1 (CHI3L1), also called cartilage glycoprotein 39 or YKL-40 in human and breast regression protein 39 in mice, is a 40 kDa chitin-binding glycoprotein without chitinase activity, and it has been shown to act as an important regulator of acute and chronic inflammation.[1]
This molecule is synthesized under inflammatory conditions, including bronchial asthma, inflammatory bowel disease, and cancer, but is not highly expressed under physiological conditions. This glycoprotein is expressed and secreted by a variety of cell types including articular chondrocytes, synoviocytes, osteoblasts, macrophages, neutrophils, and epithelial cells.[2]
Up to now, several studies have shown an important link between CHI3L1 and inflammation or metabolic diseases, including asthma, hypertension, diabetes mellitus, insulin resistance, and atherosclerosis, and naturally believe that CHI3L1 may be a potential biomarker and therapeutic target for the related diseases.[1]
It is also highly regulated, being stimulated by a number of mediators including IL-13 and IFN-γ and being detected in exaggerated quantities in the circulation and or biologic fluids from patients with a spectrum of diseases including asthma, COPD, rheumatoid arthritis, cancer, diabetes, and atherosclerosis. YKL-40/Chi3l1 is elevated in the serum of patients with community-acquired pneumonia requiring hospitalizations, patients with cystic fibrosis and acute lung infections, and control subjects following endotoxin injection.[3]

<u>Clinical use and areas of investigation:</u>
Immune Response, Infection and Inflammation

4. TEST PRINCIPLE

In the BioVendor Human Chitinase 3-Like 1 ELISA, standards and samples are incubated in microplate wells pre-coated with anti-human CHI3L1 antibody. After 120 minutes incubation and washing, biotin-labelled polyclonal anti-human CHI3L1 antibody is added and incubated with captured CHI3L1 for 120 minutes. After another washing, streptavidin-horseradish peroxidase conjugate is added. After 20 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 CHI3L1. 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	2x20 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 CHI3L1 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 CHI3L1 in the stock solution is **4 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	4 ng/ml
250 µl of 4 ng/ml	250 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 0.5 ng/ml	250 µl	0.25 ng/ml
250 µl of 0.25 ng/ml	250 µl	0.125 ng/ml

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

Stability and storage:

Standard stock solution (4 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 CHI3L1 in serum and plasma (EDTA, citrate, heparin).

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 plasma samples just prior to the assay 300x with Dilution Buffer in two steps as follows:

Dilution A (30x):

Add 5 µl of sample into 145 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

Dilution B (10x):

Add 15 µl of Dilution A into 135 µl of Dilution Buffer to prepare final dilution (300x). **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 **2 hours**, 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 **2 hours**, 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 **20 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 **20 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 CHI3L1 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 4**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 2**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 1**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 0.5**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	**Standard 0.25**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	**Standard 0.125**	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 CHI3L1 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. 1 ng/ml (from standard curve) x 300 = 300 ng/ml.

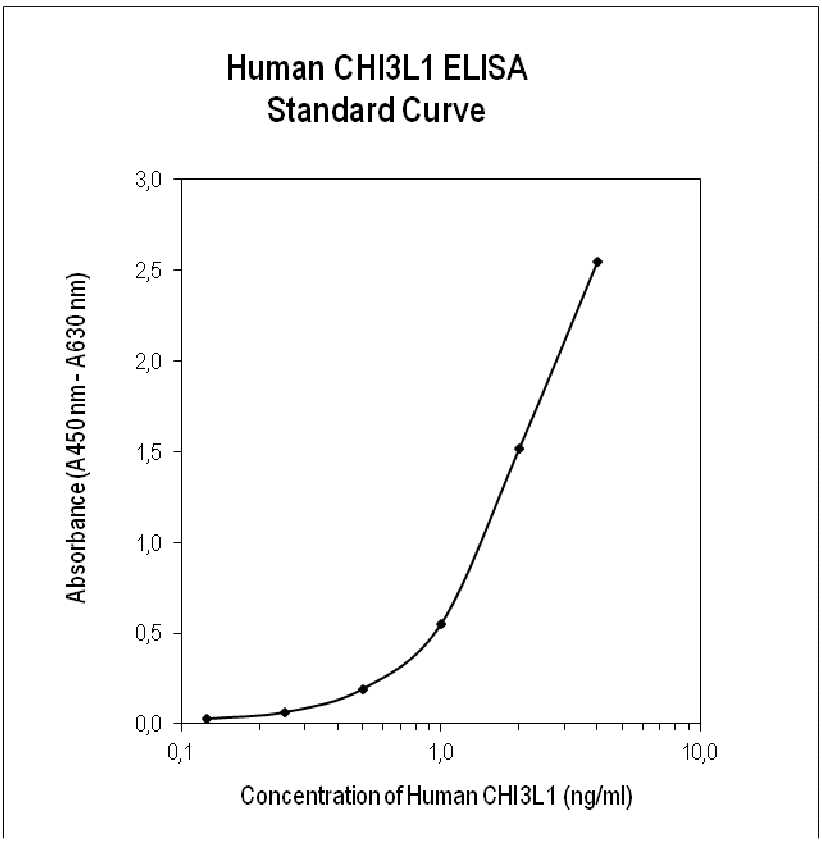


Figure 2: Typical Standard Curve for Human CHI3L1 ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human CHI3L1 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 Mouse myeloma cell line (NSO-derived) is used as the Master Standard in this assay. The CHI3L1 is a 41.3 kDa protein consisting of 362 amino acids, with a C-terminal 6-His tag.

15. PRELIMINARY POPULATION AND CLINICAL 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 CHI3L1 ELISA in our laboratory.

n	CHI3L1				
	Mean	Median	SD	Min	Max
80	103.7	76.5	139.2	14.0	1247.0

16. REFERENCES

» References to CHI3L1:

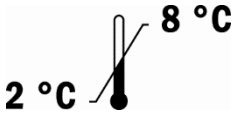
1 Gong Z. Increased expression of chitinase 3-like 1 in aorta of patients with atherosclerosis and suppression of atherosclerosis in apolipoprotein E-knockout mice by chitinase 3-like 1 gene silencing. Mediators Inflamm. 2014;2014:905463

2 Libreros S. Exploring the role of CHI3L1 in "pre-metastatic" lungs of mammary tumor-bearing mice. Front Physiol. 2013;4:392

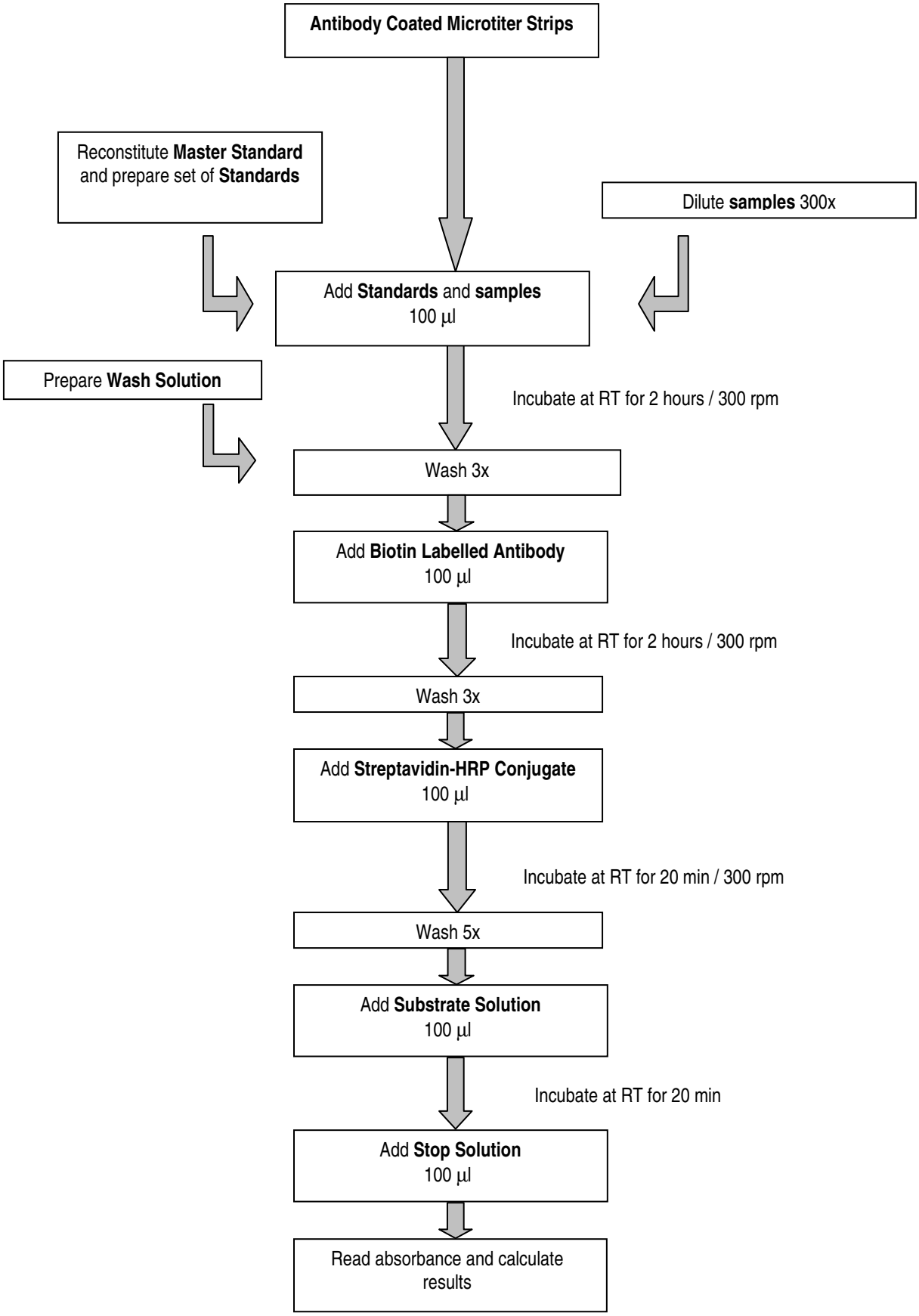
3 Dela Cruz CS. Chitinase 3-like-1 promotes Streptococcus pneumoniae killing and augments host tolerance to lung antibacterial responses. Cell Host Microbe. 2012;12(1):34-46

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

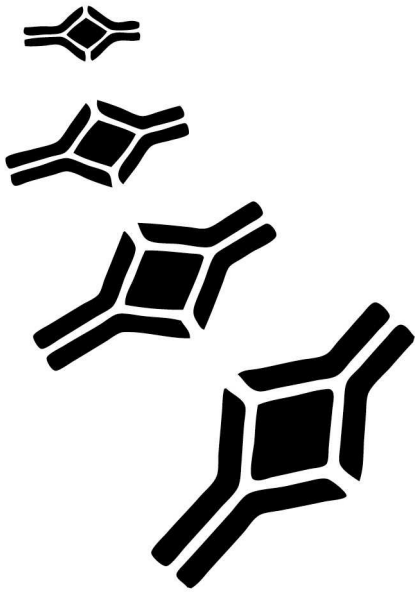
17. EXPLANATION OF SYMBOLS

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												
B												
C												
D												
E												
F												
G												
H												



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