Human Cardiotrophin-1 ELISA

Cat. No.: RD192026200R

Manufacturer

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

1. Intended Use

The RD192026200R Human Cardiotrophin-1 ELISA is a sandwich enzyme immunoassay using two specific antibodies for the quantitative measurement of human Cardiotrophin-1 in human **EDTA plasma** and **tissue culture medium**. It is intended for *in vitro* and research use only.

Features

- The total assay time is less than four hours.
- The kit measures total Cardiotrophin-1.
- Calibrator is recombinat protein based.
- Quality Controls are recombinant protein based.

2. Storage, Expiration

Store the kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

3. Summary

Cardiotrophin-1 (CT-1) is a 201 amino acid member of the interleukin-6 superfamily. It was identified by its ability to induce hypertrophic response in cardiac myocytes. CT-1 mRNA levels were found both in cardiac myocytes and in cardiac nonmyocytes. CT-1 was also detected in abundance in normal adult human lung and was expressed in both fetal and adult airway smooth muscle cells.
CT-1activates gp130 dependent signaling and stimulates the Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway to transduce hypertrophic and cytoprotective signals in cardiac myocytes. CT-1 has also a neurotrophic function. CT-1 deficiency causes increased motoneuron cell death in spinal cord and brainstem nuclei of mice during a period between embryonic day 14 and the first postnatal week. Moreover, CT-1 is a hepatocyte survival factor that efficiently reduces hepatocellular damage in animal models of acute liver injury. CT-1 expression is augmented after hypoxic stimulation and it can protect cardiac cells when added either prior to simulated ischaemia or at the time of reoxygenation following simulated ischaemia. CT-1 can induce expression of the protective heat shock proteins (hsps) in cardiac cells. CT-1 increases ventricular expression of ANP, brain natriuretic peptide (BNP) and angiotensinogen mRNA. CT-1 levels are significantly elevated in patients with heart failure, patients with dilatative cardiomyopathy, moderate/severe mitral regurgitation, stable and unstable angina and after acute myocardial infarction.

4. Test Principle

In the BioVendor's Human Cardiotrophin-1 ELISA, calibrators or quality controls or samples are incubated in microtitration wells coated with a polyclonal anti-human Cardiotrophin-1 antibody. After two-hours incubation and washing, biotin-labelled monoclonal anti-human Cardiotrophin-1 antibody is added and incubated with captured Cardiotrophin-1. After a thorough wash, 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 H_2O_2-tetramethylbenzidine. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of Cardiotrophin-1. A standard curve is constructed by plotting absorbance values versus concentrations of Cardiotrophin-1 Calibrator, and concentrations of unknown samples are determined using this standard curve.

5. Precautions

- For *in vitro* and research use only.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide. Wear gloves and eye protection when handling these reagents. In case of contact with the Stop Solution and the Substrate Solution wash skin thoroughly with water and seek medical attention, if irritation persists.
- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- The materials must not be pipetted by mouth.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- Reagents with different lot numbers should not be mixed.
- Do not use kit reagents beyond the expiration date indicated on the box label.

6. Reagents Supplied

Cat. No.	*Kit Components*	*Quantity*
C141111	Microtiter Plate coated with capture Anti- Cardiotrophin-1 Antibody, sealed	96 wells
C142611	Biotin Labelled Anti- Cardiotrophin-1 Antibody, ready to use	13 ml
C142351	Streptavidin-Horseradish Peroxidase Conjugate, ready to use	13 ml
C143141	Cardiotrophin-1 Master Calibrator, lyophilized	1 ng
C144171	Quality Control High, lyophilized	1 vial
C144271	Quality Control Low, lyophilized	1 vial
C005114	Dilution Buffer, ready to use	20 ml
C006121	Wash Solution Concentrate (10x)	100 ml
C007111	Substrate Solution (TMB), ready to use	13 ml
C008111	Stop Solution (0.2 M H_2SO_4), ready to use	13 ml
-	Instruction Manual + Certificate of Analysis	1 pc

7. Materials Required but Not Supplied

- Test tubes for sample dilution

- Precision pipettes to deliver 10-1000 µl and disposable tips
- Multichannel pipette 50-200 µl and disposable tips
- Microplate reader with 450 ± 10 nm filter
- Software package facilitating data generation and analysis
- Orbital microplate shaker capable of approximately 300 rpm (optional)
- Microtitration plate washer (optional). Manual washing is possible but not preferable.
- Absorbent material for blotting the microtiter plate
- Glassware (graduated cylinder and bottle for Wash Solution)
- Deionized (distilled) water

8. Preparation of Reagents

All reagents need to be brought to room temperature prior to the assay.

- If you do not use the whole plate, return unused strips in the provided aluminium bag with desiccant and seal the bag carefully. Keep the unused strips at 2-8°C, protected from the moisture.

Preparation of reagents for 1 plate:

Wash Solution
Dilute 100 ml of Wash Solution concentrate with 900 ml of deionized (distilled) water.

Stability and storage:
The diluted Wash Solution is stable for one month when stored at 2-8°C.

CT-1 Calibrators

Reconstitute Cardiotrophin-1 Master Calibrator with 1 ml of Dilution Buffer. The concentration of the human Cardiotrophin-1 in the stock solution is 1ng/ml. Prepare Calibrators as follows:

Calibrator volume	*Dilution Buffer*	*Concentration*
stock	-----	1 ng/ml
200 ul of stock	800 µl	200 pg/ml
500 ul of std.200 pg/ml	500 µl	100 pg/ml
500 ul of std.100 pg/ml	500 µl	50 pg/ml
500 ul of std. 50 pg/ml	750 µl	20 pg/ml
500 ul of std. 20 pg/ml	500 µl	10 pg/ml
500 ul of std. 10 pg/ml	500 µl	5 pg/ml

Dilute Calibrators 1:1 with Dilution Buffer prior to use, e.g. 150 µl of Calibrator + 150 µl of Dilution Buffer.

Stability and storage:
Reconstituted Calibrators are stable until the expiration date (see label on the box) when stored at -20°C.
Do not store the diluted (1:1) Calibrators.

Quality Controls
Reconstitute Quality Controls with 250 µl of Dilution Buffer. Refer to the Certificate of Analysis for actual Quality Control values.
Dilute Quality Controls 1:1 with Dilution Buffer prior to use, e.g. 125 µl of Quality Control + 125 µl of Dilution Buffer.

Stability and storage:
Reconstituted Quality Controls are stable until the expiration date (see label on the box) when stored at -20°C.
Do not store the diluted (1:1) Quality Control.

9. Preparation of Samples

Dilute samples 1:1 with Dilution Buffer prior to use, e.g. 150 µl of sample + 150 µl of Dilution Buffer.

Stability and storage:
Samples should be stored at -20°C, preferable at -70°C.
Do not store the diluted (1:1) samples.

10. Assay Procedure

1) Pipet 100 µl of diluted Calibrators (5-200 pg/ml), Dilution Buffer (=Blank), Quality Controls 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 5-times with Wash Solution (0.35 ml per well).
4) Add 100 µl of Biotin Labelled Anti-Cardiotrophin-1 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 5-times with Wash Solution (0.35 ml per well).
7) Add 100 µl of Streptavidin-HRP Conjugate.
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. (Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.)
11) Incubate the plate at room temperature for 15 minutes, the incubation time may be extended up to 20 minutes (e.g., if the reaction temperature is below than 20°C). No shaking!
12) Stop the colour development by adding 100 µl of Stop Solution.
13) Determine the absorbance by reading the plate at 450 nm. The absorbance should be read within 5-15 minutes following step 12.

Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine Cardiotrophin-1 concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

	strip 1+ 2	**strip 3 + 4**	**strip 5+ 6**	**strip 7+ 8**	**strip 9+10**	**strip 11+ 12**
A	Calibrator 200	QC Low	Sample 8	Sample 16	Sample 24	Sample 32
B	Calibrator 100	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Calibrator 50	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Calibrator 20	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Calibrator 10	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Calibrator 5	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	Blank	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	QC High	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of work sheet.

11. Calculations

Most microtiter plate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the absorbance at 450 nm (Y) of Calibrators versus *log* of the known concentration (X) of Calibrators, using the four-parameter function.

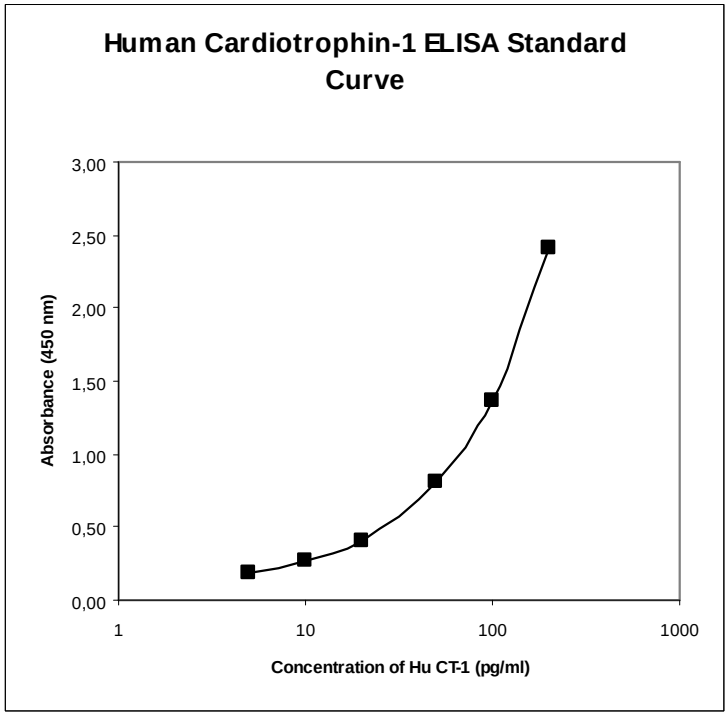


Figure 2: Typical Standard Curve for Cardiotrophin-1.

Alternatively, the *logit log* function can be used to linearize the standard curve (i.e. *logit* of absorbance (Y) is plotted versus *log* of the known concentration (X) of Calibrators).

Samples, Quality Controls and Calibrators are diluted 1:1 prior to analysis, so there is no need to account for this dilution.

12. Limits of Assay

Results exceeding Cardiotrophin-1 level of 200 pg/ml should be repeated with more diluted samples. In this case, dilution factors need to be taken into consideration when calculating the Cardiotrophin-1 concentrations.

13. Performance Characteristics

This chapter presents typical analytical data of BioVendor Human Cardiotrophin-1 ELISA.
For actual Standard Curve see the Certificate of Analysis.

- **Sensitivity**

The limit of detection (defined as human Cardiotrophin-1 concentration giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is defined as follows:

Analytical Limit of Detection is calculated from the real Cardiotrophin-1 values in wells and is 1pg/ml
Assay Sensitivity takes the dilution of samples into consideration and is calculated according to the formula:
Assay Sensitivity = Analytical Limit of Detection x sample dilution = 1pg/ml x 2 = 2 pg/ml

*Dilution Buffer is pipetted into blank wells.

- **Specificity**

The antibodies used in the Human Cardiotrophin-1 ELISA kit are specific to human Cardiotrophin-1, with no detectable crossreactivities to the following blood components:
IL-6
No crossreactivity was found to the following animal sera: mouse, rabbit, goat, rat, bovine, horse, sheep and pig.

- **Precision**

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

Sample	*Mean (pg/ml)*	*SD*	*CV (%)*
1	30.7	1.4	4.7
2	17.4	1.5	9
3	4.6	0.2	4

Inter-assay (Run-to-Run) (n=4)

Sample	*Mean (pg/ml)*	*SD*	*CV (%)*
1	30.7	1.4	4.7
2	17.4	1.5	9
3	4.6	0.2	4

- **Spiking Recovery**

Samples were spiked with varying amounts of human Cardiotrophin-1, diluted with Dilution Buffer and assayed.

Sample	***O**bserved (pg/ml)*	***E**xpected (pg/ml)*	*Recovery **O/E** (%)*
1	21.8	-	-
	32.2	31.8	102
	38.3	41.8	91.7
	44.4	51.8	86.2
2	30.8	-	-
	38.5	40.8	94.4
	47.6	50.8	93.7
	52.3	60.8	86.0

- **Dilution Linearity**

Samples (diluted 1:1 with Dilution Buffer) were further serially diluted with Dilution Buffer (see table below) and assayed.

Sample	*Dilution*	***O**bserved (pg/ml)*	***E**xpected (pg/ml)*	*Recovery **O/E** (%)*
1	-	83.3	-	-
	2x	40.6	41.6	97.6
	4x	19.0	20.8	91.3
	8x	8.6	10.4	82.6
2	-	45.2	-	-
	2x	22.4	22.6	99.1
	4x	9.9	11.3	87.6
	8x	4.4	5.5	80.0

14. Definition of Master Calibrator and Quality Controls

A recombinant protein is used as Calibrator and Quality Controls. The recombinant Cardiotrophin-1 is a 21,5 kDa protein containing 201 amino acid residues and was produced in E. coli.

15. Troubleshooting and FAQs

1/ 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

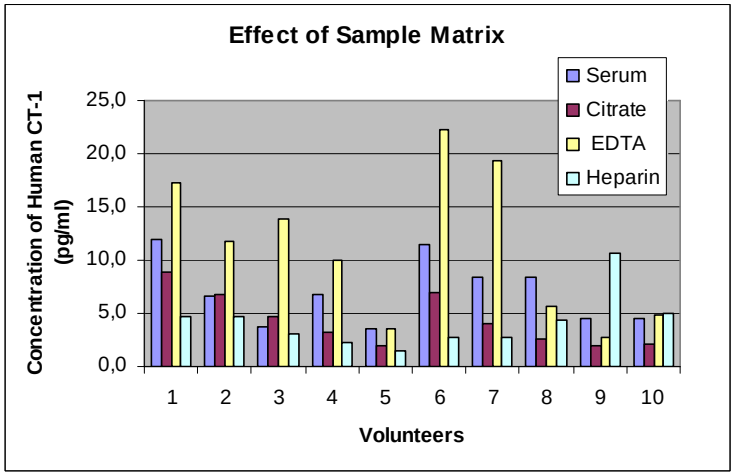
2/ High signal and background in all wells
Possible explanations:
· Improper or inadequate washing
· Overdeveloping; incubation time should be decreased before addition of Stop Solution

3/ High coefficient of variation (CV)
Possible explanation:
· Improper or inadequate washing

4/ Effect of freezing/thawing on the concentration of human Cardiotrophin-1 in EDTA plasma
No significant decline was observed in concentration of human Cardiotrophin-1 in EDTA plasma samples after repeated (5x) freezing/thawing cycles.

Number of freeze / thaw cycles	Volunteer		
	A (pg/ml)	B (pg/ml)	C /pg/ml)
1x	34,1	10,8	11,7
3x	29,8	10,8	10,8
5x	31,7	9,5	10,6
10x	28,2	9,4	10,1

5/ Effect of sample matrix (serum/plasma)
Samples from 10 healthy individuals were taken and treated by different methods (serum, citrate plasma, EDTA plasma and heparin plasma), results shown below:



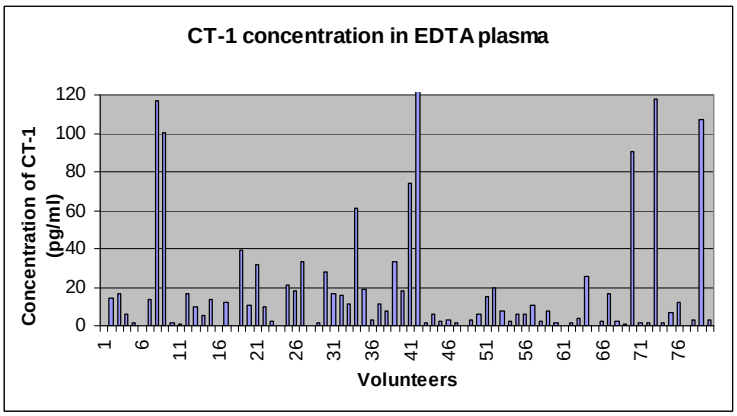
Volunteer	Serum (pg/ml)	Plasma (pg/ml)		
		Citrate	EDTA	Heparin
1	11,9	8,8	17,3	4,7
2	6,6	6,7	11,8	4,7
3	3,7	4,6	13,95	3,1
4	6,8	3,3	10	2,2
5	3,6	2,0	3,5	1,4
6	11,5	6,9	22,3	2,7
7	8,4	4	19,3	2,8
8	8,4	2,6	5,6	4,3
9	4,54	1,9	2,7	10,7
10	4,5	2,1	4,8	5

Mean values of human Cardiotrophin-1 in serum, citrate plasma, EDTA plasma and heparin plasma, are reported in the table below:

Sample (n=10)	Mean (pg/ml)	Plasma/Serum (%)
Serum	6,9	-
Citrate Plasma	4,3	62
EDTA Plasma	11,1	161
Heparin Plasma	4,2	61

6/ EDTA plasma level of CT-1

Values of human Cardiotrophin-1 in EDTA plasma were measured for 80 volunteers:



7/ Stability of samples at 4°C

Samples should be stored at -20°C. However, no decline was observed in concentration of Cardiotrophin-1 in samples when stored at 4°C for 2 days. To avoid microbial contamination, add NaN3 to a final concentration 0,1% to the samples.

Temperature, time	Volunteer		
	A (pg/ml)	B (pg/ml)	C (pg/ml)
Sample at -20°C	27,9	107,8	92,2
Incubation at 4°C, 12 hours	30,0	105,3	85,5
Incubation at 4°C, 3 days	32,4	104,1	91,2

8/ Why are calibrator solutions diluted 2x prior to use in ELISA and what does it mean for calculation of results?

The dilution buffer suppresses the matrix effect of samples. It is a common practice to use the same dilution for samples and calibrators because of simple calculation of results: concentrations of samples can be read directly off the curve without considering a dilution factor. Thus, in Human Cardiotrophin-1 ELISA the same dilution of 1:1 is used for calibrators and plasma/serum samples.

16. References

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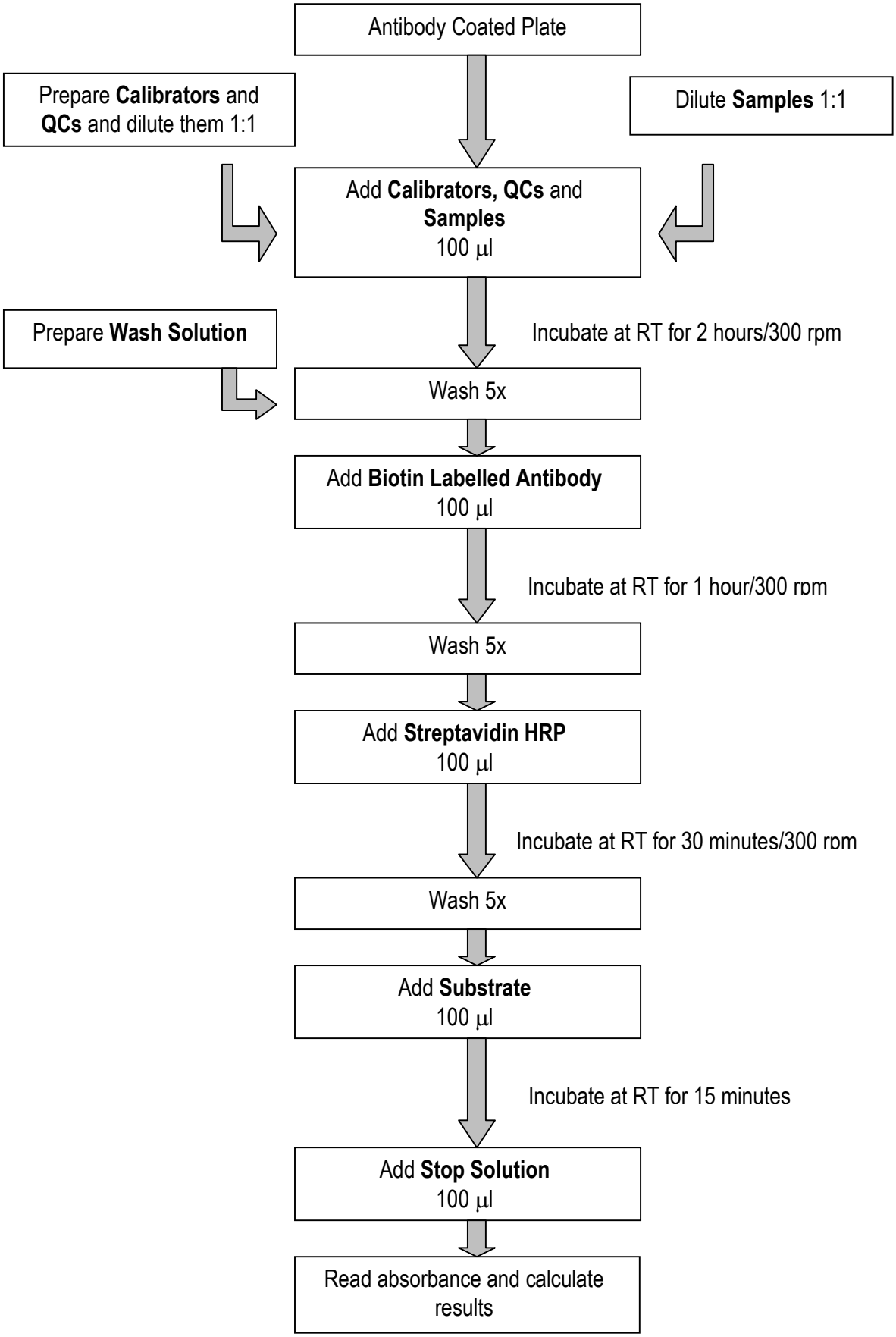
For more references on this product
see our WebPages at www.biovendor.com

Assay Procedure Summary



	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Notes: