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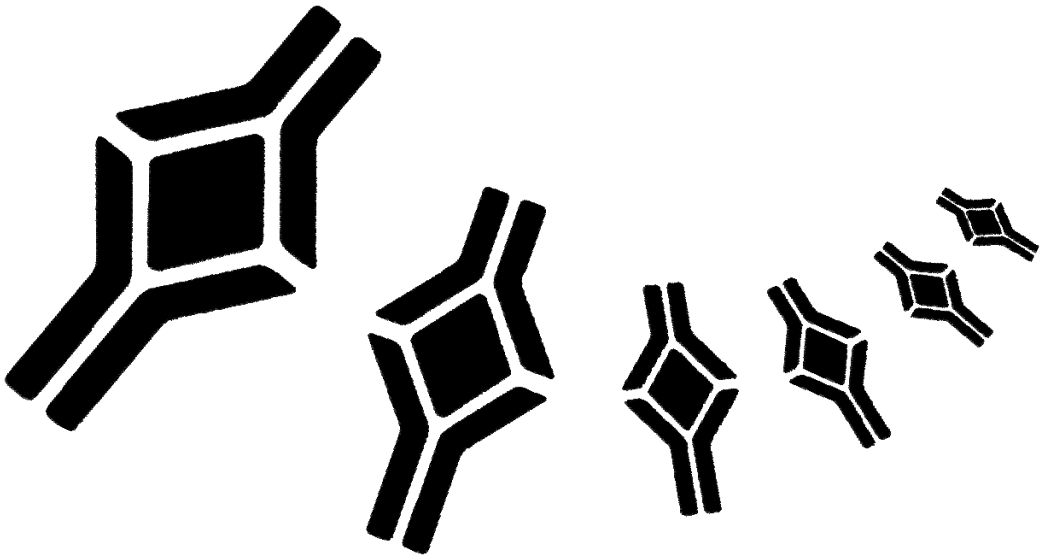

HUMAN DICKKOPF-RELATED PROTEIN 1 ELISA

Product Data Sheet

Cat. No.: RD191207200R

For Research Use Only

VERSION 99 100610 18

CONTENTS

1.	INTENDED USE	3
2.	STORAGE, EXPIRATION	3
3.	INTRODUCTION	4
4.	TEST PRINCIPLE	5
5.	PRECAUTIONS	5
6.	TECHNICAL HINTS	6
7.	REAGENT SUPPLIED	6
8.	MATERIAL REQUIRED BUT NOT SUPPLIED	7
9.	PREPARATION OF REAGENTS	7
10.	PREPARATION OF SAMPLES	9
11.	ASSAY PROCEDURE	10
12.	CALCULATIONS	11
13.	PERFORMANCE CHARACTERISTICS	13
14.	DEFINITION OF THE STANDARD	16
15.	METHOD COMPARISON	16
16.	TROUBLESHOOTING AND FAQS	17
17.	REFERENCES	18
18.	EXPLANATION OF SYMBOLS	19

» 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 RD191207200R Human Dickkopf-Related Protein 1 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Dickkopf-Related Protein 1 (Dkk-1).

Features

- It is intended for research use only
- The total assay time is less than 3.5 hours
- The kit measures Dkk-1 in serum
- Assay format is 96 wells
- Quality Controls are human serum based
- 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

Dickkopf-1 (Dkk-1) is encoded by the gene *dickkopf* as are other members of the dickkopf protein family in vertebrates, Dkk-2, -3, -4 and a distant family member *soggy,* also called *Dickkopf-like protein 1 (DKKL1).* The Dickkopf name is derived from the german dick=thick and kopf=head, and the overexpression of Dkk-1 in *Xenopus* induces ectopic head formation and bilocation. Inhibitition of Dkk-1 by anti-Dkk-1 antibodies leads to microcephaly. This protein was independently discovered and characterised and named "Sk". Dkk-1/Sk is composed of 266 amino acids and has a predicted molecular weight of 25.8kDa. The protein has one N-glycosylation site at the N-terminus.

Dkk proteins possess a signal sequence and have two typical cysteine-rich domains. The second cysteine-rich domain is required for binding to known receptors Lrp6 and Kremen-2.

Dkks modulate Wnt signalling. Their effect is mostly inhibitory with the exception of Dkk-2 which activates Wnt-signalling. Wnt signalling pathways are involved in cell proliferation, cell identity and cell polarity from embryonic to adult homeostasis. Wnt forms a complex composed of a seven-transmembrane receptor Frizzled (Fz) receptor and a lipoprotein-receptor related protein (Lrp5 or Lrp6). This triple complex formation results in stabilization of beta-catenin, increasing the amount of beta-catenin entering the nucleus and, thus, activating the pathway. Dkk-1 inhibits this process by its interaction with Lrp6 which blocks Wnt-Fz-Lrp complex formation. The Lrp6-Dkk-1 complex enters the cell by endocytosis via the coreceptor Kremen.

Dkk-1 in mouse was found to be expressed in bone, specifically in osteoblasts and osteocytes. Dkk-1 inhibition of Wnt signaling can result in decreased bone density.

In mouse models lacking Dkk-1, the result was incomplete development of structures anterior to the midbrain resulting in perinatal death. Limb development in Dkk-1 null mice results in syndactyly and polydactyly. Excess expression of Dkk-1 may result in pathological bone loss in such diseases as osteoarthritis, multiple myeloma, Paget's disease, and osteoporosis.

The Dkk-1 ELISA allows quantification of Dkk-1 in serum or plasma and, thus, can be used for research on cancer, especially bone and lung cancer, as well as in Paget's disease or in the problems with bone calcification.

<u>Areas of investigation:</u>

Oncology
(multiple myeloma, lung, breast and prostate cancer)
Bone metabolism
(osteoarthritis, osteoporosis, Paget´s disease)

4. TEST PRINCIPLE

In the Biovendor Human Dickkopf-Related Protein 1 ELISA, standards, quality controls and samples are incubated in microtitration wells pre-coated with polyclonal anti-human Dkk-1 antibody. After a 60 minutes incubation followed by washing, biotin labelled polyclonal anti-human Dkk-1 antibody is added and incubated with the captured Dkk-1 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 Dkk-1. A standard curve is constructed by plotting absorbance values against Dkk-1 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 human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. These materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- 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 Conc. (100x)	concentrated	0.13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	20 ml
Biotin-Ab Diluent	ready to use	13 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
- 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 stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate
Dilution Buffer
Biotin-Ab Diluent
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 Dkk-1 Master Standard:
IMPORTANT: 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 Dkk-1 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 stock	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:
Do not store the diluted Standard solutions.

Quality Controls HIGH, LOW
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Controls concentrations!!!
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Reconstituted Quality Controls are ready to use, do not dilute them.
Stability and storage:
Do not store the reconstituted Quality Controls.

Biotin Labelled Antibody Conc. (100x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) with 99 parts Biotin-Ab Diluent.

Example: 10 µl of "Biotin Labelled Antibody Concentrate (100x)" + 990 µl of Biotin-Ab Diluent for 1 strip (8 wells).
Stability and storage:
Opened Biotin Labelled Antibody Concentrate (100x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Biotin Labelled Antibody solution.

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 Dkk-1 in serum.

Samples should be assayed immediately after collection or should be stored at -20°C or -70°C. 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.

Preparation of samples:
Dilute samples 3x with Dilution Buffer just prior to the assay (e.g. 50 µl of sample + 100µl of Dilution Buffer for singlets, or preferably 100 µl of sample + 200 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 for long-term storage. Avoid repeated freeze/thaw cycles.
Do not store the diluted samples.

See Chapter 13 for stability of serum samples if stored at 2-8°C and effect of freezing/thawing on the concentration of human Dkk-1.

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, Quality Controls, 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 5-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 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 Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for 30 min, 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 Dkk-1 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 4	Blank	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	QC High	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
H	QC Low	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 absorbance (Y) of Standards against the known concentration (X) of Standards, using the four-parameter algorithm. Results are reported as concentration of Dkk-1 (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. 0.75 ng/ml (from standard curve) x 3 (dilution factor) = 2.25 ng/ml.

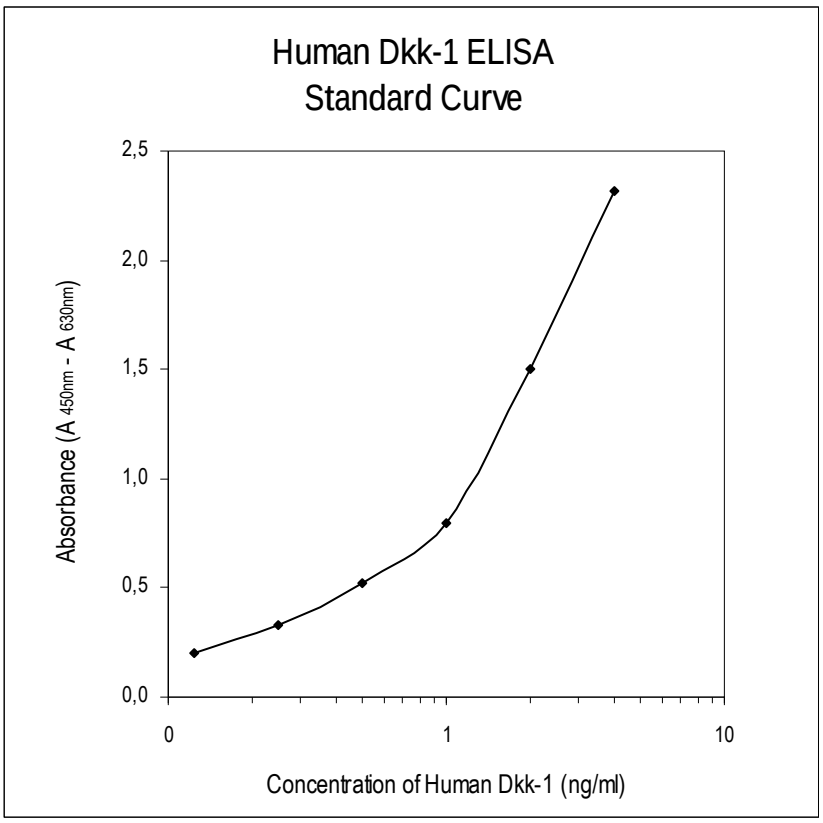

Figure 2: Typical Standard Curve for Human Dickkopf-Related Protein 1 ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human Dickkopf_Related Protein 1 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 Dkk-1 values in wells and is: 0.01 ng/ml.

* Dilution Buffer is pipetted into Blank wells.

- Limit of Assay

Results exceeding human Dkk-1 level of 4 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the Dkk-1 concentration.

- Specificity

The antibodies used in this ELISA are specific for human Dkk-1.

Sera of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com

Mammalian serum Sample	*Observed crossreactivity*
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	no
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

» Presented results are multiplied by respective dilution factor

- Precision

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	6.29	0.300	3.5
2	3.49	0.205	5.9

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	2.56	0.152	5.6
2	1.821	0.156	8.6

- Spiking Recovery

Serum samples were spiked with different amounts of human Dkk-1 and assayed.

Sample	*Observed (ng/ml)*	*Expected (ng/ml)*	*Recovery O/E (%)*
1	2.19	-	-
	6.19	6.19	100.1
	3.88	4.19	92.5
	2.96	3.19	92.6
2	3.29	-	-
	6.81	7.29	93.4
	4.82	5.29	91.2
	4.18	4.29	97.3

- Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	*Observed (ng/ml)*	*Expected (ng/ml)*	*Recovery O/E (%)*
1	-	3.62	-	-
	2x	1.69	1.81	93.6
	4x	0.84	0.91	93.2
	8x	0.43	0.45	94.5
2	-	3.49	-	-
	2x	1.63	1.79	93.4
	4x	0.77	0.87	88.3
	8x	0.42	0.44	96.1

- Stability of samples stored at 2-8°C

Samples should be stored at -20°C. However. no significant decline in concentration of human Dkk-1 was observed in serum samples after 7 days when stored at 2-8°C. To avoid microbial contamination. samples were treated with ε-aminocaproic acid and thimerosal, resulting in the final concentration of 0.03% and 0.1%. respectively.

Sample	Incubation Temp. Period	Serum *(ng/ml)*
1	-20°C	1.11
	2-8°C, 1 day	1.29
	2-8°C, 7 days	1.26
2	-20°C	1.69
	2-8°C, 1 day	1.52
	2-8°C, 7 days	1.74
3	-20°C	1.49
	2-8°C, 1 day	1.90
	2-8°C, 7 days	1.73

- Effect of Freezing/Thawing

No significant decline was observed in concentration of human Dkk-1 in serum samples 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	Serum *(ng/ml)*
1	1x	3.01
	3x	3.53
	5x	2.97
2	1x	2.63
	3x	2.62
	5x	3.01
3	1x	3.20
	3x	3.62
	5x	3.31

- 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 Dkk-1 levels with the assay.

14. DEFINITION OF THE STANDARD

The recombinant human Dkk-1 is used as the Standard. The human Dkk-1 is 25.8 kDa protein expressed in Spodoptera frugiperda (Sf 21) insect cells.

15. METHOD COMPARISON

The Biovendor Human Dickkopf-Related Protein 1 ELISA was not compared to the other commercial immunoassays.

16. 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. Quality Controls or samples

17. REFERENCES

» References to Dkk-1 protein:

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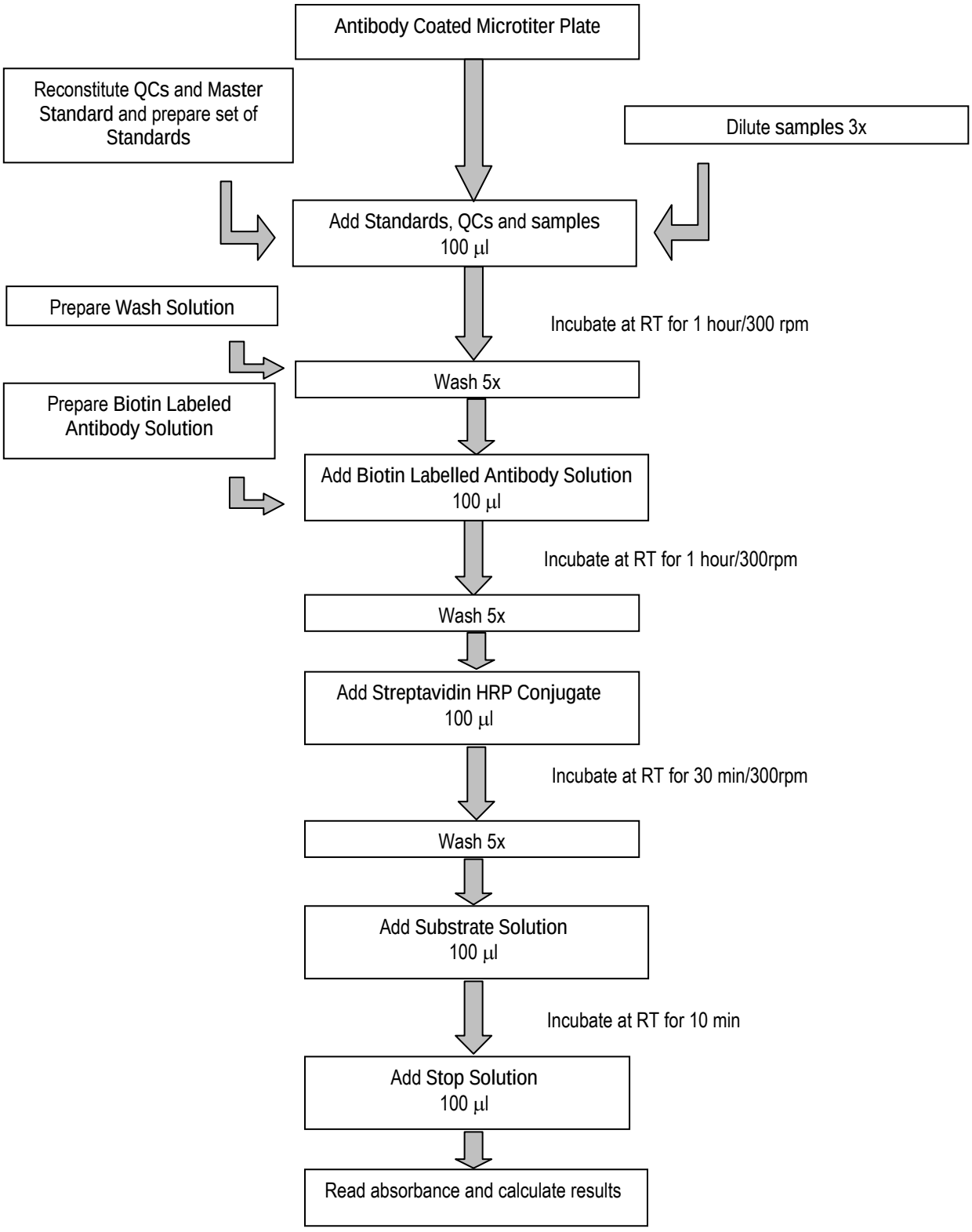
» For more references on this product see our WebPages at www.biovendor.com

18. 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

Assay Procedure Summary



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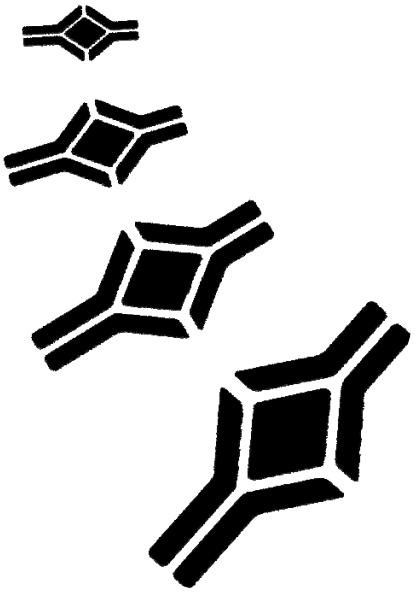

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