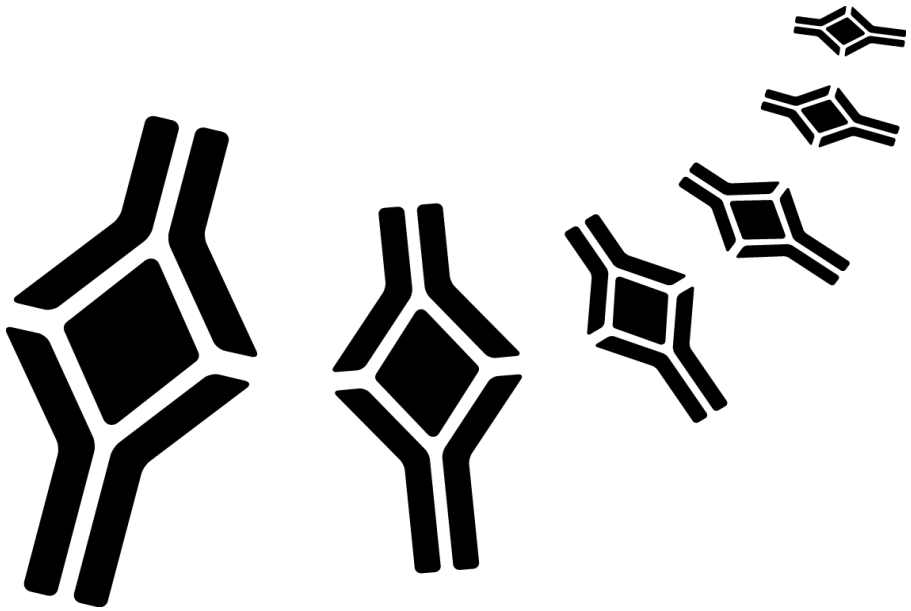
BioVendor
Research
and Diagnostic Products



HUMAN DICKKOPF-RELATED PROTEIN 1 ELISA

Product Data Sheet

Cat. No.: RD191207200R

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 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
- 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* together with other members of dickkopf protein family in vertebrates, Dkk-2, -3, -4 and a distant family member *soggy,* sometimes also called *Dickkopf-like protein 1 (DKKL1).* Dickkopf name is derived from german dick=thick and kopf=head and this protein was independently found and characterised and named as „Sk". Dkk-1/Sk is composed from 266 amino acids and has predicted molecular weight 25.8kDa. The protein has one N-glycosylation site at the N-terminus.
Dkk proteins possess the signal sequence and have two typical cysteine rich domains. The second cysteine-rich domain is required for binding to Lrp6 and Kremen-2. Up to now known receptors are from the family Lrp (Lrp5/6) and Kremen 1 and 2.
Dkks are modulating Wnt signalling. Their effect is mostly inhibitory with exception of the Dkk-2 which is activating Wnt-signalling. Wnt signalling pathways are shifting the cell proliferation, cell identity and cell polarity from embryonic to adult homeostasis. Wnt is forming the complex composed of seven-transmembrane receptor Frizzled (Fz) receptor and a lipoprotein-receptor related protein Lrp5 or Lrp6. This triple complex formation is stabilizing the beta-catenin and activating the pathway. Dkk-1 is inhibiting this process by its interaction with Lrp6 that is blocking the Wnt-Fz-Lrp complex formation. The Lrp6-Dkk-1 is entering the cell by endocytosis allowed by the coreceptor Kremen.
Dkk-1 in mouse was found to be exprimed in bone, specifically in osteoblasts and osteocytes.
The overexpression of the Dkk-1 in *Xenopus* is causing the ectopic head formation and blocation by anti-Dkk-1 antibodies lead to microcephaly. In mouse models, when missing the Dkk-1, there was found incomplete development of structures anterior to the midbrain and consequently this lead to the perinatal death. In limb development of Dkk-1 null mice there has been found syndactyly and polydactyly.
Dkk-1 ELISA is estimating the Dkk-1 in serum or plasma thus can be used in the cancer research, especially bone and lung cancer, as well as in the Paget's disease or in the problems with bone calcification.

<u>Areas of investigation:</u>
Oncology
(multiople 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 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 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. REAGENT SUPPLIED

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody	lyophilized	2 vials
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Biotin-Ab Diluent	ready to use	13 ml
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 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
Biotin-Ab Diluent
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 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.

Biotin Labelled Antibody
IMPORTANT: Refer to the Certificate of Analysis for current volume of Biotin-Ab Diluent needed for reconstitution of Biotin Labelled Antibody!!!
Reconstitute the lyophilized Biotin Labelled Antibody with Biotin-Ab Diluent just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Dilute reconstituted Biotin Labelled Antibody Concentrate (100x) with Biotin-Ab Diluent e.g. 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Biotin-Ab Diluent for 1 strip (8 wells).
Stability and storage:
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, e.g. 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.

Dilute samples 3x with Dilution Buffer just prior to the assay (e.g. 50 µl of sample + 100 µl of Dilution Buffer when assaying samples as 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 when 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, 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 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 2	Sample 10	Sample 18	Sample 26	Sample 34
B	**Standard 2**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	**Standard 1**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	**Standard 0.5**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	**Standard 0.25**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	**Standard 0.125**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
G	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40
H	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41

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 in logarithmic scale, 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.

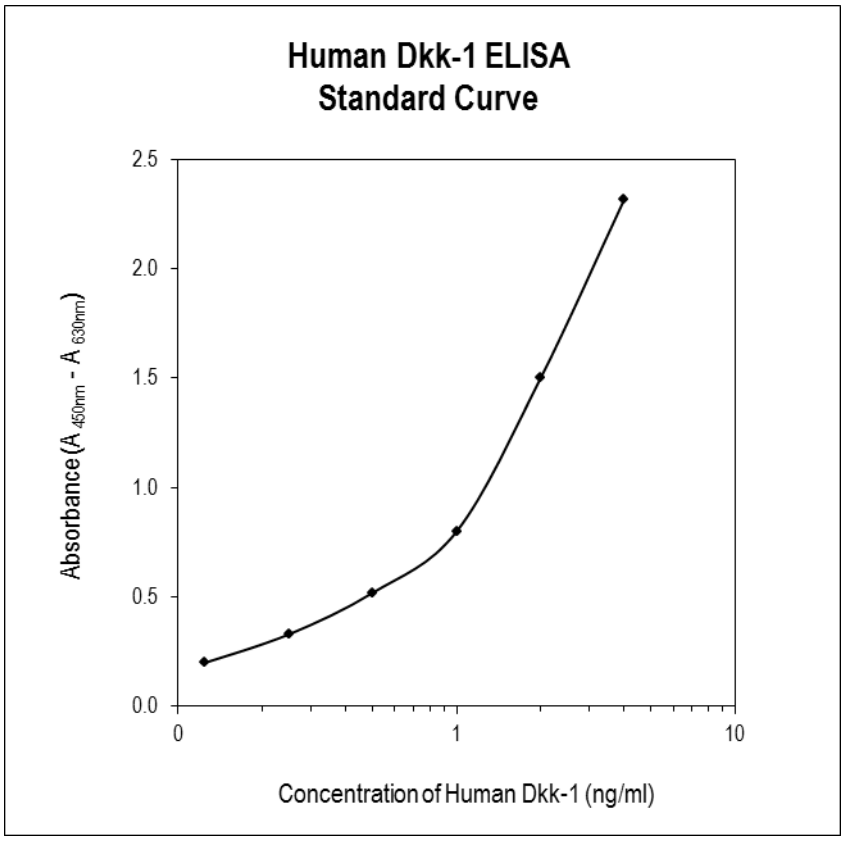


Figure 2: Typical Standard Curve for Human Dickkopf-Related Protein 1 ELISA.

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: 0.032 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	yes
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	0.30	4.8
2	3.49	0.21	5.9

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	2.56	0.15	5.6
2	1.82	0.16	8.6

- **Spiking Recovery**

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

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

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (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 sodium azide. 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

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. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 153 unselected donors (90 men + 63 women) 21-65 years old were assayed with the BioVendor Human Dickkopf-Related Protein 1 ELISA in our laboratory.

» Presented results are multiplied by respective dilution factor

- **Age dependent distribution of Dkk-1**

Sex	*Age (years)*	*n*	*Dkk-1 (ng/ml)*				
			Mean	*Median*	*SD*	*Min*	*Max*
Men	21-29	17	2.38	2.21	0.92	1.18	4.49
	30-39	26	2.45	2.36	0.79	1.14	4.21
	40-49	31	2.90	2.60	1.20	1.86	8.28
	50-65	16	2.29	2.30	0.54	1.42	3.35
Women	22-29	10	3.15	3.39	0.78	1.48	4.18
	30-39	25	2.66	2.60	0.61	1.50	3.79
	40-49	20	2.68	2.50	0.69	1.60	4.71
	50-61	8	2.57	2.08	1.31	1.58	5.86

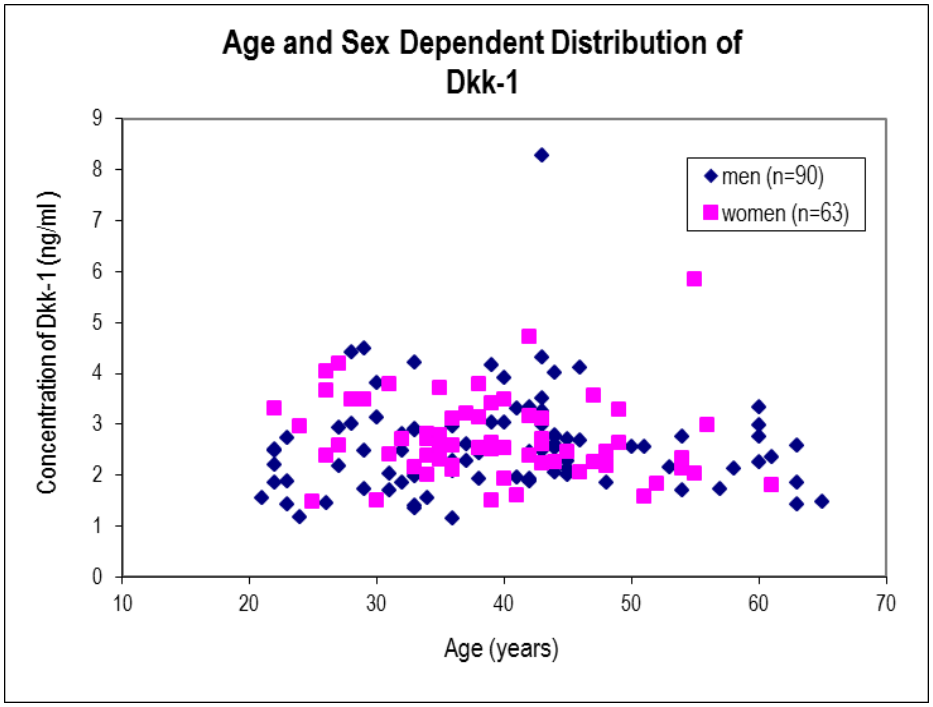


Figure 3: Human Dkk-1 concentration plotted against donor age and sex.

- **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.

16. METHOD COMPARISON

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

17. 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

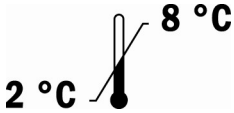
18. REFERENCES

›› References to Dkk-1 protein:

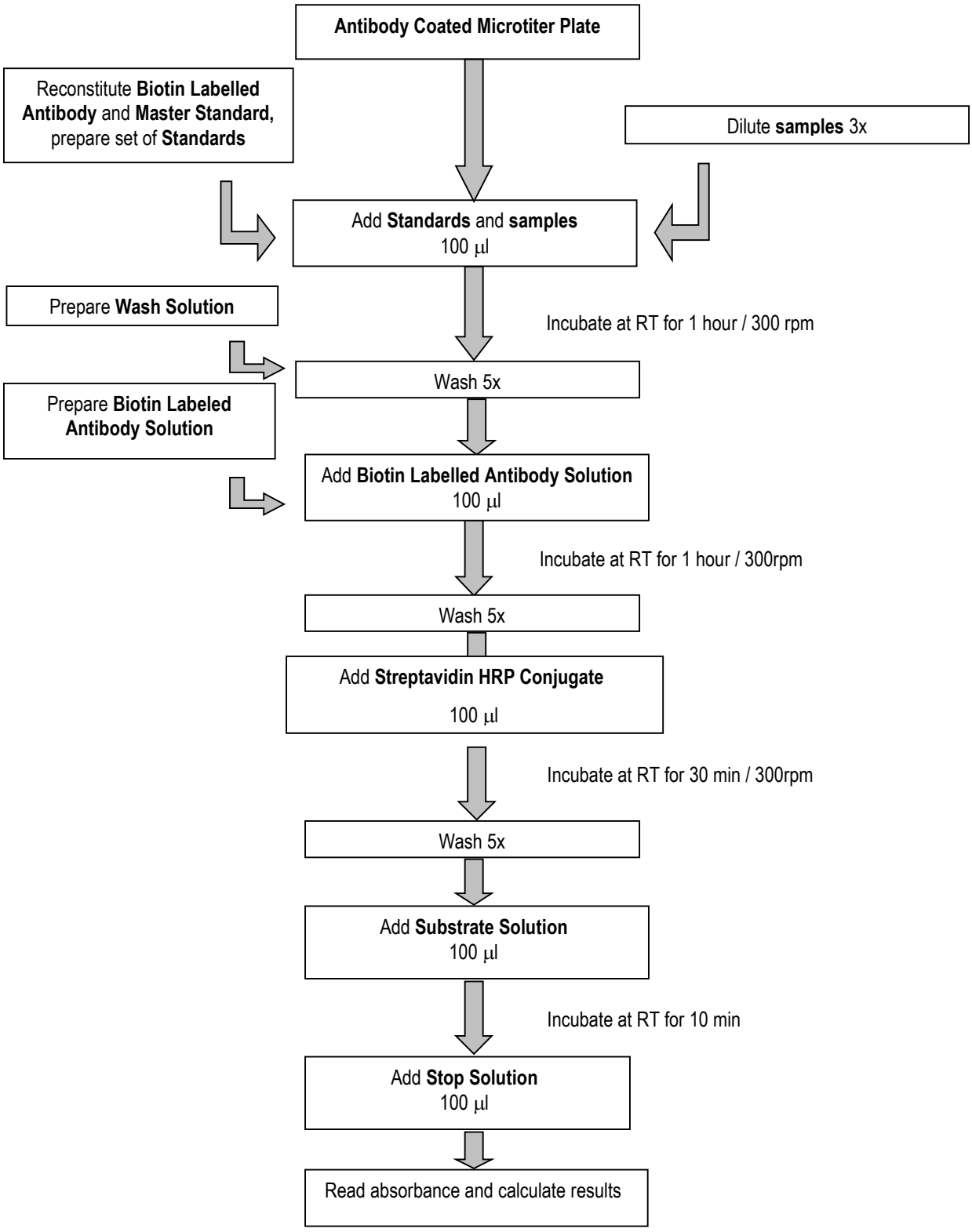
- Marshall MJ. Evans SF. Sharp CA. Powell DE. McCarthy HS. Davie MWJ: Increased circulating Dickkopf-1 in Paget´s disease of bone. Clin Biochem 42: 965-969 (2009)
- Sheng SL. Huang G. Yu B and Qin WX: Clinical Significance and Prognostic Value of Serum Dickkopf-1 Concentration in Patients with Lung Cancer. Clin Chem 55:9 1656-1664 (2009)
- Voorzanger-Rousselot N. Journe F. Doriath V. Body JJ. Garnero P: Assessment of Circulating Dickkopf-1 with a New Two-Site Immunoassay in Healthy Subjects and Women with Breast Cancer and Bone Metastases. Calcif Tissue Int 84:348-354 (2009)
- Lee N. Smolarz AJ. Olson S. David O. Reiser J. Kutner R. Daw NC. Prockop DJ. Horwitz EM and Gregory CA: A potential role od Dkk-I in the pathogenesis of osteosarcoma predicts novel diagnostic and treatment strategies. British Journal of Cancer 97:1552-1559 (2007)
- Yamabuki T. Takano A. Hayama S. Ishikawa N. Kato T. Miyamoto M. Ito T. Ito H. Miyagi Y. Nakayama H. Fujita M. Hosokawa M. Tsuchiya E. Kohno N. Kondo S. Nakamura Y and Daigo Y: Dickkopf-1 a Novel Serologic and Prognostic Biomarker for Lung and Esophageal carcinomas. Cancer Res (2007)
- Glass DA II and Karsenty G: Minireview: In Vivo Analysis of Wnt Signaling in Bone. Endocrinology 148(6):2630-2634 (2007)
- Niehrs C: REVIEW: Function and biological roles of the Dickkopf family of WNT modulators. Oncogene 25. 7469-7481 (2006)
- MacDonald BT. Adamska M and Meisler MH: Hypomorphic expression of Dkk1 in the doub leridge mouse: dose dependence and compensatory interactions with LRP6. Development 131. 2543-2552 (2004)
- Fedi P. Bafico A. Soria AN. Burgess WH. Miki T. Bottaro DP. Kraus MH and Aaronson SA: Isolation and Biochemical Characterisation of the Human Dkk-1 Homologue. a Novel Inhibitor of Mammalian Wnt Signaling. J Biol Chem274:27 19465-19472 (1999)

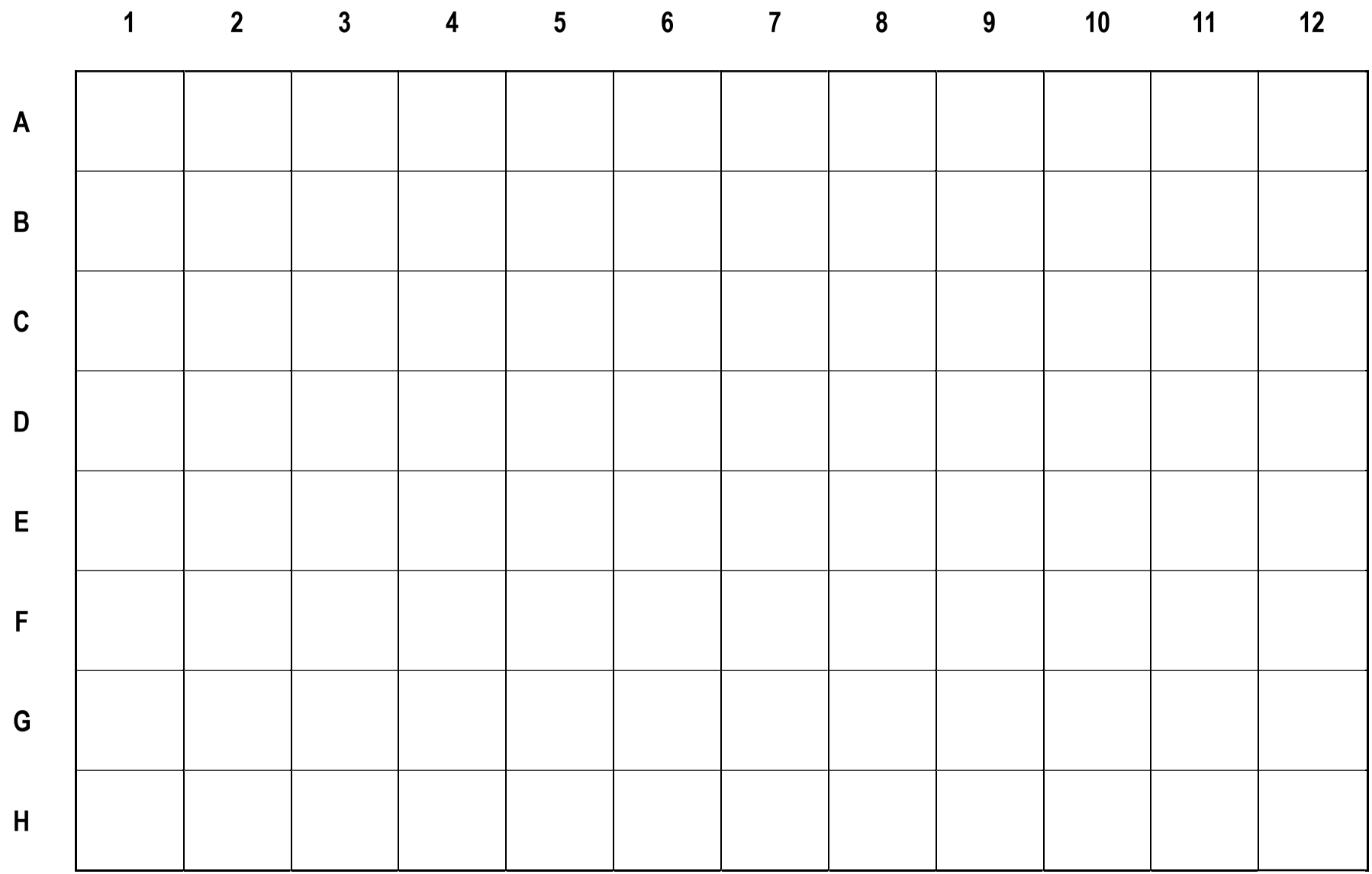
›› For more references on this product see our WebPages at www.biovendor.com

19. 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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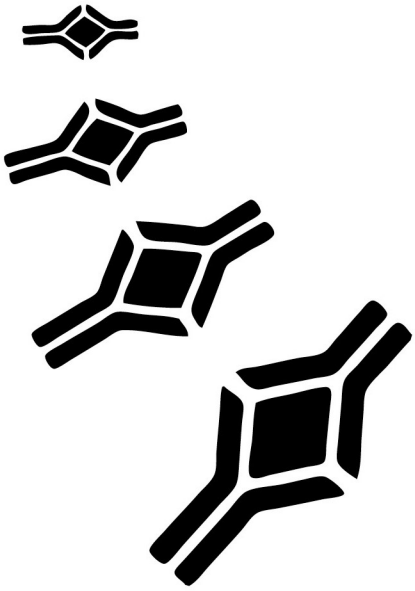

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