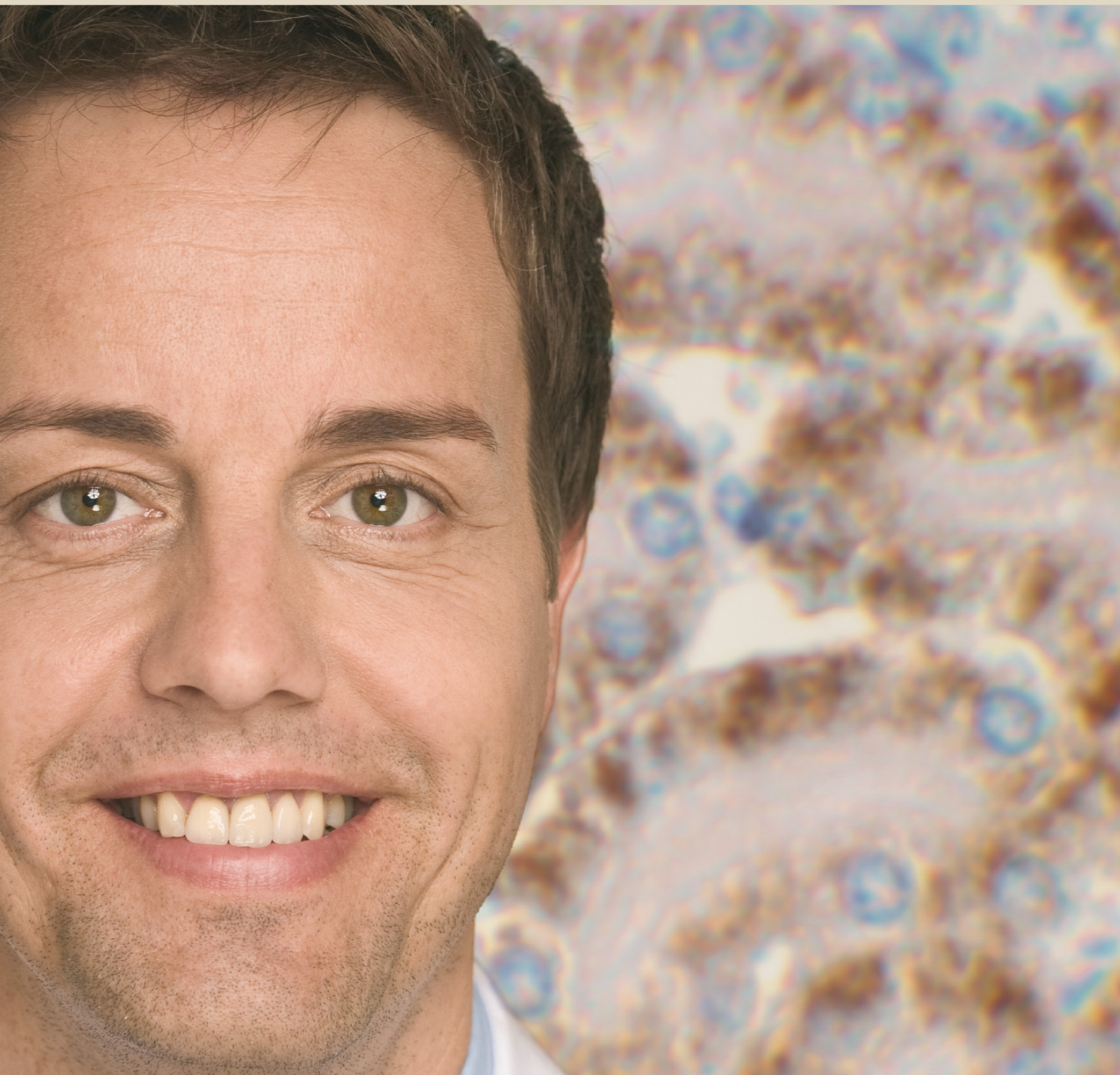


Human DKK-1 ELISA Kit



Instruction Manual

PromoKine

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Intended Use

This PromoKine Assay is intended for the quantitative determination of human DKK-1 in serum. It is for *in vitro* research use only.

Introduction

Dickkopf-1 (DKK-1) is a 28,672 Da secreted protein that acts as soluble inhibitor of the WNT signalling pathway. This pathway contains lipid-modified glycoproteins that activate cell surface receptor-mediated signal transduction to regulate cell activities like: cell fate, proliferation, migration, polarity and gene expression. DKK-1 regulates developmental processes of all kinds. Thus, DKK-1 is also involved in the regulation of bone metabolism as it effects osteoblast differentiation and in regulation of tumourgenetic activity.

Possible Indications

- Osteoarthritis
- Osteoporosis
- Colon Carcinomas
- Multiple Myelomas
- Bone Metastases

Material Supplied

Content	Kit Components	Quantity
PLATE	anti human DKK-1 pre-coated microtiter strips in strip holder	12 x 8 tests
WASHBUF	Wash buffer concentrate 20x, natural cap	1 x 50 ml
ASYBUF	Assay buffer, red cap, ready to use	1 x 40 ml
AB	biotinylated anti DKK-1 antibody, green cap, lyophilised	2 x lyophilised
STD	Standards, (0; 3.125; 6.25; 12.5; 25; 50 pmol/l), white caps, lyophilised	6 vials lyophilised
CTRL	Control, yellow cap, lyophilised, exact concentration after reconstitution see label	1 vial lyophilised
CONJ	Conjugate, (streptavidin-HRPO), amber cap, ready to use	1 x 22 ml
SUB	Substrate (TMB solution), blue cap, ready to use	1 x 22 ml
STOP	STOP solution, white cap, ready to use	1 x 7 ml

- 2 self-adhesive plastic films
- Instruction manual for use, QC protocol

Material Required but not Supplied

- 1.5 ml reaction vials
- Precision pipettes calibrated to deliver 50-1000 µl and disposable tips
- ELISA reader for absorbance at 450 nm (or from 450 nm to 620 nm), Graph paper or software for calculation of results
- Plate washer is recommended for washing
- Distilled or deionised water

Reagents and Sample Preparation

The assay has been validated for the use of serum samples only.

Cell culture measurements are possible. An additional buffer and assay protocol is available on request.

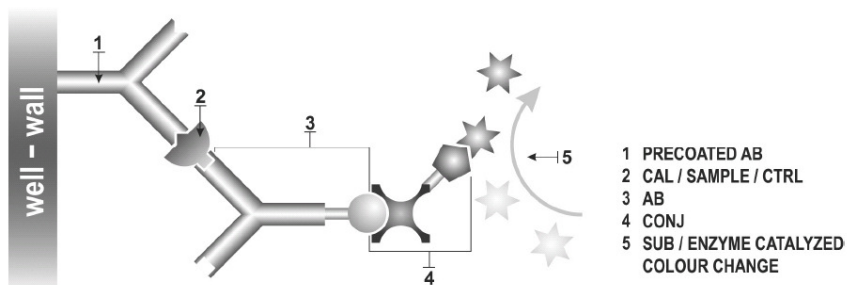
DKK-1 has a good stability in whole blood and serum, showing a mean loss after 24hrs at RT of 19% and 13% respectively.

Serum samples should be stored aliquoted at -20°C or -70°C. Avoid freeze-thaw cycles. 5 freeze-thaw cycles decreased the signals by approx. 23%. EDTA and Heparin plasma show significantly lower levels than serum. Lipemic or hemolyzed samples may give erroneous results. Samples should be mixed well before assaying.

Reconstitution / Handling:

- Samples:** Samples must be diluted 1:4 prior to use in the assay. Dilute samples 1:4 (1+3) in assay buffer (e.g 50 µl sample +150 µl assay buffer). We recommend duplicates for all values.
- STD (Standards) and CTRL (Control):** Each in 400 µl deionised or distilled water at room temperature (18-26°C) for 15 minutes. Reconstituted STD and CTRL are stable at -20°C until expiry date on label, avoid more than 3 freeze-thaw cycles.
- One vial of AB (Antibody)** is reconstituted in 11 ml assaybuffer and handled exactly like STD & CTRL. The whole amount of reconstituted antibody must be used within one run because the diluted antibody is not stable.
- WASHBUF (Wash buffer):** Dilute the concentrate 1:20: e.g. 50 ml WASHBUF + 950 ml distilled water. Crystals in the buffer concentrate will dissolve at room temperature. The diluted buffer is stable at 2-8°C until expiry date stated on label. Use only diluted WASHBUF (Wash buffer) for the assay performance:

Principle of the Assay



Assay Protocol

- All reagents and samples must be at room temperature (18-26°C) before they can be used in the assay.
- Mark position for BLK/STD/SAMPLE/CTRL (Blank/Standard/Sample/Control) on the protocol sheet.
- Take microtiter strips out of the alu bag, reserve a minimum of one well as Blank. Store unused strips with desiccant at 2-8°C in the alu bag. Strips are stable until expiry date stated on the label.
- Add 50 µl STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective wells, except blank.
- Add 200 µl AB (biotinylated anti DKK-1 antibody) into each well, except blank, swirl gently.
- Cover tightly and incubate over night (18-24h) at room temperature (18-26°C) in the dark.
- Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer), remove remaining WASHBUF by hitting plate against paper towel after the last wash.

- Add 200 µl CONJ (Conjugate) into each well.
- Cover tightly and incubate for 1 hour at room temperature (18-26°C) in the dark.
- Aspirate and wash wells 5x with 300 µl diluted WASHBUF (Wash buffer), remove remaining WASHBUF by hitting plate against paper towel after the last wash.
- Add 200 µl SUB (Substrate) into each well
- Incubate for 30 minutes at room temperature (18-26°C) in the dark.
- Add 50 µl STOP (Stop solution) into each well.
- Measure absorbance immediately at 450 nm with reference 620 nm, if available.

Calculation of Results

Subtract the blank extinction from all other values. Construct the standard curve from the standard values. Use commercially available software or graph paper. Obtain sample concentration from this standard curve. The assay has been evaluated using a 4PL algorithm. Different curve fitting methods need to be evaluated by the user. The concentration of the samples will be calculated from the standard curve. The dilution factor 1:4, or other respective dilution factors, must be considered for the calculation of results.

The quality control protocol supplied with the kit shows the results of the final release QC for each kit. Data for optical density obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life. However, this does not affect validity of results as long as an optical density of 1.50 is obtained for the standard with the highest concentration.

Assay Characteristics

Reference data:	Serum samples of 18 blood donors had a mean DKK-1 level of 52.6 pmol/l (median 45.7 pmol/l, SD 20 pmol/l) Each laboratory should establish its own reference data.
Standard range:	0; 3.125; 6.25; 12.5; 25; 50 pmol/l
Conversion:	1 pmol/l = 28.68 pg/ml
Sample volume:	50 µl human serum
Detection Limit:	(0 pmol/l + 3 SD): 0.38 pmol/l
Incubation time:	Overnight / 1 h / 30 min

Precision

Inter-Assay (n=10)

Mean (pmol/l)	71.2	159.6
SD	6.2	20
CV%	9%	12%

Intra-Assay (n=16)

Mean (pmol/l)	19.1	10.1
SD	1.2	0.8
CV%	7%	8%

Note: SAMPLE VALUES WERE CORRECTED FOR DILUTION

Technical Hints

- Do not mix or substitute reagents with those from other lots or sources.
- Do not mix stoppers and caps from different reagents or use reagents between lots.
- Do not use reagents beyond expiration date. Protect reagents from direct sunlight.
- Substrate solution should remain colourless until added to the plate.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.

Precautions

All test components of human source were tested with 3rd generation tests against HIV-Ab and HBsAg and were found negative. Nevertheless, they should be handled and disposed as if they were infectious.

All liquid reagents contain 0.1% Proclin 300 as preservative. Proclin 300 is not toxic in concentrations used in this kit. It may cause allergic skin reactions, avoid contact with skin or eyes.

- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Avoid all contact with the reagents by using gloves. Flush immediately with water if contact occurs.

Literature

- Dickkopf-1 is a master regulator of joint remodeling. D. Diarra et al., Nature medicine 2007 Feb;Vol 2 no 2
- Serum concentration of Dickkopf-1 protein are increased in patients with multiple myelomas and reduced after autologous stem cell transplantation. M.C. Politou et al., Int. J. of Cancer 2006 Oct 1;119(7): 1728-31
- Mechanism of bone metastasis; G.D Roodmann, N Engl J Med (2004); 350:1655-64
- WNT signalling in osteoblasts and bone disease. J.J. Westerdorf et al., Gene (2004) 341;19-39
- A functional genomics approach for the identification of putative tumor suppressor gene. Dickkopf-1 as suppressor of Hela cell transformation. A.M. Mikheev et al., Carcinogenesis (2004) pp 47-59

Ordering Information

Product Name	Product Description	Size	Catalog Number
DKK-1 ELISA Kit, human	Human DKK-1 (Dickkopf-1) ELISA Kit	96 Tests	PK-EL-KB20412

***For in vitro research use only.
Not for diagnostic or therapeutic procedures.***

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