DKK-1

(EN) ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF HUMAN DKK-1 IN SERUM
CAT. NO. BI-20413. 12 X 8 TESTS

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

rev.no. 220524 (replacing 190108)

This kit was developed and manufactured by:
Biomedica Medizinprodukte GmbH, A-1210 Wien, Divischgasse 4
Tel. +43/1/291 07 45, Fax +43/1/291 07 6389, E-mail info@bmgrp.com

CONTENT / INHALT

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1) INTRODUCTION

Dickkopf-1(DKK-1) is a 28.67 kDa 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.

Areas of Interest:

- Rheumatoid arthritis
- Diffuse idiopathic skeletal hyperostosis
- Monoclonal gammopathy of undetermined significance
- Oncology
- Myeloma bone disease
- Ankylosing spondylitis
- Glucocorticoid induced osteoporosis

2) CONTENTS OF THE KIT

CONT	KIT COMPONENTS	QUANTITY
PLATE	Mouse monoclonal anti human DKK-1 antibody pre-coated microtiter strips in strip holder packed in aluminum bag with desiccant	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 10 ml
AB	biotinylated DKK-1 antibody, green cap, ready to use	1 x 7 ml
STD	Standards 1-6 (0, 10, 20, 40, 80, 160 pmol/l), white caps, lyophilised	6 vials
CTRL	Control, yellow cap, lyophilised, exact concentration after reconstitution see label	1 vial
CONJ	Conjugate (streptavidin-HRPO), amber cap, ready to use	1 x 13 ml
SUB	Substrate (TMB solution), blue cap, ready to use	1 x 13 ml
STOP	STOP solution, white cap, ready to use	1 x 7 ml

3) ADDITIONAL MATERIAL IN THE KIT

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

4) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 20 µl, 50 µl, 100 µl, 200 µl and disposable tips
- ELISA reader for absorbance at 450 nm (or from 450 nm to 630 nm)
- Graph paper or software for calculation of results
- Plate washer is recommended for washing, alternative multichannel pipette or manifold dispenser
- Distilled or deionised water

5) REAGENTS AND SAMPLE PREPARATION

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

All reagents as originally supplied in the kit are stable at 4°C (2-8°C) until expiration date stated on the label of each reagent.

Sample preparation:

Collect venous blood samples by using standardized blood collection tubes for serum. Allow samples to clot for 30 minutes at room temperature before performing serum separation by centrifugation, e.g. 20 min at 2000 x g, preferably at 4°C (2-8°C).

Measure the acquired samples immediately or aliquot samples in polypropylene tubes and store at -25°C or lower. Avoid more than three freeze-thaw cycles. Lipemic or haemolysed samples may give erroneous results. Samples should be mixed well before assaying.

If samples read higher than STD6, we recommend to dilute serum samples with STD1 and to test again.

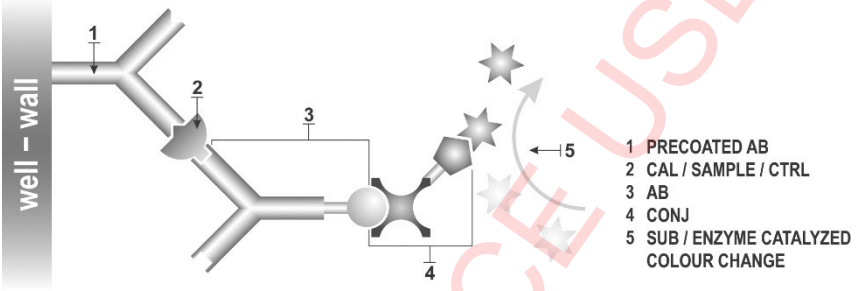
For further information on sample stability please visit our website www.bmgrp.com (Validation Data) or contact our customer service by e-mail info@bmgrp.com or by phone +43/ 1/ 29107-45.

Reconstitution/Handling:

STD (Standards) and CTRL (Control): Add 200 µl deionised or distilled water into each vial, reconstitute at room temperature (18-24°C) for 15 min. Swirl gently. Take care of complete dissolving of lyophilisate. The reconstituted STDs and CTRL shall be stored at -25°C or lower until expiry date stated on the label. **Avoid more than one freeze-thaw cycle.**

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 4 °C (2-8 °C) for one month. Use only diluted WASHBUF (Wash buffer) for the assay performance.

6) PRINCIPLE OF THE ASSAY:



7) ASSAY PROTOCOL

All reagents and samples must be at room temperature (18-24°C) before they can be used in the assay.
Mark position for STD/SAMPLE/CTRL (Standard/Sample/Control) on the protocol sheet.
Take microtiter strips out of the aluminum bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminum bag. Strips are stable until expiry date stated on the label.

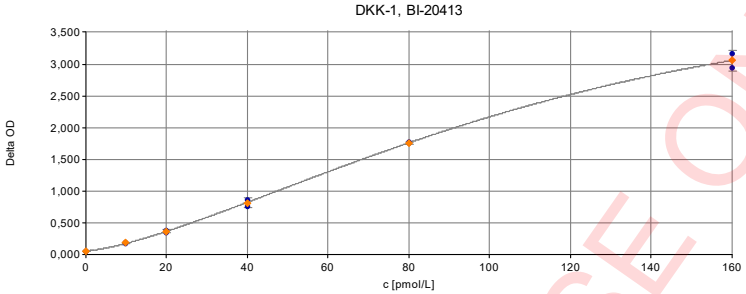
1. Pipette 50 µl ASYBUF (assay buffer) into all wells.
2. Add 20 µl STD/CTRL/SAMPLE (standards/control/sample) in duplicate into respective wells, swirl gently.
3. Add 50 µl AB (biotinylated DKK-1 antibody) into each well, swirl gently.
4. **Cover tightly and incubate for 2 hours at room temperature (18-24°C).**
5. 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.
6. Add 100 µl CONJ (conjugate) into each well.
7. Cover tightly and incubate for 1 hour at room temperature (18-24°C).
8. 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.
9. Add 100 µl SUB (substrate) into each well.
10. Incubate for 30 min at room temperature (18-24°C) in the dark.
11. Add 50 µl STOP (stop solution) into each well, swirl gently.
12. Measure absorbance immediately at 450 nm with reference 630 nm, if available.

8) CALCULATION OF RESULTS

Read the optical density (OD) of all wells on a plate reader using 450 nm wavelength (correction wavelength 630 nm). Construct the standard curve from the OD values of the STD. Use commercially available software or graph paper. Obtain sample concentration from this standard curve. The assay was evaluated with 5PL algorithm. Different curve fitting methods need to be evaluated by the user.

Respective dilution factors have to be considered when calculating the final concentration of the sample.

Example typical STD-curve:



The quality control (QC) protocol supplied with the kit shows the results of the final release QC for each kit at production date. Data for OD 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 OD of 1.50 or more is obtained for the STD with the highest concentration and the value of the CTRL is in range (target range see label).

9) ASSAY CHARACTERISTICS

Method	Sandwich ELISA, 96-well strip plate, HRP/TMB			
Sample type	Serum			
Standard range	0-160 pmol/l (0, 10, 20, 40, 80, 160 pmol/l), = 0-4103 pg/ml			
Conversion factor	1 pg/ml = 0.039 pmol/l (MW = 25.8 kDa)			
Sample volume	20 µl human serum			
Incubation time, temp.	DAY TEST 2 h / 1 h / 30 min, room temperature (18-24°C)			
Sensitivity	LOD: 1.7 pmol/l (0 pmol/l + 3 SD) , LLOQ: 1.25 pmol/			
Specificity	oligomeric forms of natural and recombinant human DKK-1.			
Cross-reactivity	Human only, No cross-reactivity or interference with recombinant human DKK-4, Kremen-1, Kremen-2 or LRP-6 is observed.			
Precision	Intra-assay (n=5) ≤ 3%, Inter-assay (n=9) ≤ 3%			
Spike/Recovery (average recovery spiked with 40 pmol/l rec. DKK-1)	Serum (n=8) = 92%			
Dilution linearity (average recovery of expected DKK-1 after a 1+1; 1+3; 1+7 dilution)	Dilution:	1+1	1+3	1+7
	Serum (n=4)	109%	104%	100%
Values from apparently healthy individuals	Median Serum (n=51) = 34 pmol/l Each laboratory should establish its own reference range for the samples under investigation.			

For further information on assay characteristics please visit our website www.bmgrp.com (Validation Data) or contact our customer service by e-mail info@bmgrp.com or by phone +43/ 1/ 29107-45.

10) PRECISION

Intra-assay: 2 samples of known concentrations were tested 5 times within 1 assay lot by 1 operator.

Inter-assay: 2 samples of known concentrations were tested 9 times within 2 assay lots and each in a different test assembly.

Intra-assay (n=5)	Sample 1	Sample 2
Mean (pmol/l)	19.9	80.1
SD (pmol/l)	0.5	2.7
CV (%)	3	3

Inter-assay (n=9)	Sample 1	Sample 2
Mean (pmol/l)	19.7	80.4
SD (pmol/l)	0.6	2.1
CV (%)	3	3

11) 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.

12) PRECAUTIONS

All test components of human source were tested against HIV-Ab, HCV-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 950 as preservative.
Avoid contact with skin and mucous membrane. Proclin 950 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 with water immediately after contact!

13) LITERATURE

1. Circulating Dickkopf-1 and Radiological Progression in Patients with Early Rheumatoid Arthritis Treated with Etanercept. Garnero P, Tabassi NC, Voorzanger-Rousselot N. J Rheumatol, 2008; 35(12):2313-5.
2. Serum concentrations of DKK-1 correlate with the extent of bone disease in patients with multiple myeloma. Kaiser M, Mieth M, Liebisch P, Oberlander R, Rademacher J, Jakob C, Kleeberg L, Fleissner C, Braendle E, Peters M, Stover D, Sezer O, Heider U. Eur J Haematol, 2008; 80(6):490-4.
3. Low circulating Dickkopf-1 and its link with severity of spinal involvement in diffuse idiopathic skeletal hyperostosis. Senolt L, Hulejova H, Krystufkova O, Forejtova S, Cerezo LA, Gatterova J, Pavelka K, Vencovsky J. Ann Rheum Dis, 2012; 71(1):71-4.
4. Biomarkers of Bone Metabolism in Ankylosing Spondylitis in Relation to Osteoproliferation and Osteoporosis. Klingberg E, Nurkkala M, Carlsten H, Forsblad-d'Elia H. J Rheumatol, 2014; 41: 1349-1356.
5. Bone microstructural changes revealed by high-resolution peripheral quantitative computed tomography imaging and elevated DKK1 and MIP-1α levels in patients with MGUS. Ng AC, Khosla S, Charatcharoenwitthaya N, Kumar SK, Achenbach SJ, Holets MF, McCready LK, Melton LJ, Kyle RA, Rajkumar SV, Drake MT. Blood, 2011; 118: 6529-6534.
6. High dickkopf-1 levels in sera and leukocytes from children with 21-hydroxylase deficiency on chronic glucocorticoid treatment. Brunetti G, Faienza MF, Piacente L, Ventura A, Oranger A, Carbone C, Di Benedetto A, Colaianni G, Gigante M, Mori G, Gesualdo L, Colucci S, Cavallo L, Grano M. Am J Physiol Endocrinol Metab, 2013; 304: E546-E554.
7. DKK-1 in prostate cancer diagnosis and follow up. D'Amelio P, Roato I, Oderda M, Soria F, Zitella A, Ferracini R, Mengozzi G, Gontero P, Isaia GC. BMC Clinical Pathology, 2014; 14:11.
8. Dickkopf-1 as a mediator and novel target in malignant bone disease. Rachner TD, Göbel A, Benad-Mehner P, Hofbauer LC, Rauner M. Cancer Letters, 2014; 346:172–177.

SYMBOLS

Expiry date / Verfallsdatum / Date de péremption / Data di scadenza /Fecha de caducidad / Data de validade / Uiterste gebruiksdatum / Udløbsdato / Utgångsdatum / Termin Ważności / Lejárati idő / Doba exspirácie / Doba exspirace

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In vitro Diagnostic Medical Device (for in Vitro Diagnostic Use)/ In vitro Diagnostikum (zur In-vitro-Diagnostik) / Dispositif médical de diagnostic in vitro (Pour usage diagnostique in vitro) / Dispositivo medico per diagnostica in vitro (per uso diagnostico in vitro) / Dispositivo médico de diagnóstico in vitro (para uso diagnóstico in vitro) / Dispositivo médico para diagnóstico in vitro (Para utilização de diagnóstico "in vitro") / Medisch hulpmiddel voor diagnostiek in vitro (Voor diagnostisch gebruik in vitro) / Medicinsk udstyr til in vitro-diagnostik (Udelukkende til in vitro diagnostisk anvendelse) / Medicinteknisk produkt avsedd för in vitro-diagnostik (För in vitro-diagnostiskt bruk) / Wyrób medyczny do Diagnostyki In Vitro / In vitro orvosdiagnosztikai termék / In vitro diagnostický zdravotnícky materiál (určené pre diagnostiku „in vitro“) / In vitro diagnostický zdravotnicky materiál (určeno pro diagnostiku „in vitro“)

Lot-Batch Number / Charge-Chargennummer / Lot-Code du lot / Lotto-Numero di lotto / Lote-Código de lote / Lote-Código do lote / Lot-Partijnummer / Lot-Batchkode / Lot-Satskod / Numer serii / Lot-Batch szám / Číslo šarže / Číslo šarže

Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por / Vervaardigd door / Fabrikation af / Tillverkad av / Wyprodukowane pr / Gyártotta / Vyrobené / Vyrobeno

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Store at between / Lagerung bei zwischen / Conserver à entre / Conservare a tra / Conservar a temp. entre / Armazene a entre / Bewaar bij tussen / Opbevares mellem / Förvaras vid / Przechowywać w / Tároljuk …… között / Skladujte v rozsahu / Skladujte v rozmezí

Contains sufficient for x tests / Inhalt ausreichend für x Tests / Contient suffisant pour x tests / Contenuto sufficiente per x test / Contiene suficiente para x pruebas / Contém suficiente para x testes / Bevat voldoende voor x bepalingen / Indeholder tilstrækkeligt til x prøver / Innehållet räcker till x analyser / Zawartość na x testów / Tartalma X teszt elvégzésére elegendő / Obsahuje materiál pre x testov / Obsahuje materiál pro x testů

BI-20413 DKK-1
ASSAY PROTOCOL AND CHECKLIST

PREPARATION OF REAGENTS:

- ❑ Bring all reagents to room temperature (18-24°C).
- ❑ Prepare reagents as instructed.
- ❑ Bring unused and prepared components to the storage temperature mentioned in the package insert.
- ❑ Take microtiter strips out of the aluminium bag and mark positions on the protocol sheet.

TEST PROCEDURE:

- ❑ Step 1) Pipette 50 µl ASYBUF (Assaybuffer) into each well.
- ❑ Step 2) Pipette 20 µl STD/SAMPLE/CTRL (standard/sample/control) into each well.
- ❑ Step 3) Add 50 µl AB (biotinylated anti DKK-1) into all wells, swirl gently.
- ❑ **Step 4) Cover tightly and incubate for 2 hours at RT (18-24°C).**
- ❑ Step 5) Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- ❑ Step 6) Add 100 µl CONJ (Conjugate) into each well.
- ❑ **Step 7) Cover tightly and incubate for 1 hour at RT (18-24°C).**
- ❑ Step 8) Aspirate and wash wells with 300 µl WASHBUF (Wash buffer) five times. Remove remaining buffer by hitting plate against paper towel.
- ❑ Step 9) Add 100 µl SUB (Substrate) into each well.
- ❑ **Step 10) Incubate for 30 minutes at RT (18-24°C) in the dark.**
- ❑ Step 11) Add 50 µl STOP (Stop solution) into each well.
- ❑ Step 12) Read Optical Density at 450 nm with reference 630 nm, if available.