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
CONTENT

Additional information on our products is available on our website.
Zusätzliche Information zu unseren Produkten ist auf unserer Homepage erhältlich.

www.bmgrp.com
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 tumourgenic 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

<table>
<thead>
<tr>
<th>CONT</th>
<th>KIT COMPONENTS</th>
<th>QUANTITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLATE</td>
<td>Mouse monoclonal anti human DKK-1 antibody pre-coated microtiter strips in strip holder packed in aluminium bag with desiccant</td>
<td>12 x 8 tests</td>
</tr>
<tr>
<td>WASHBUF</td>
<td>Wash buffer concentrate 20x, natural cap</td>
<td>1 x 50 ml</td>
</tr>
<tr>
<td>ASYBUF</td>
<td>Assay buffer, red cap, ready to use</td>
<td>1 x 10 ml</td>
</tr>
<tr>
<td>AB</td>
<td>biotinylated DKK-1 antibody, green cap, ready to use</td>
<td>1 x 7 ml</td>
</tr>
<tr>
<td>STD</td>
<td>Standards 1-6 (0, 10, 20, 40, 80, 160 pmol/l), white caps, lyophilised</td>
<td>6 vials</td>
</tr>
<tr>
<td>CTRL</td>
<td>Control, yellow cap, lyophilised, exact concentration after reconstitution see label</td>
<td>1 vial</td>
</tr>
<tr>
<td>CONJ</td>
<td>Conjugate (streptavidin-HRPO), amber cap, ready to use</td>
<td>1 x 13 ml</td>
</tr>
<tr>
<td>SUB</td>
<td>Substrate (TMB solution), blue cap, ready to use</td>
<td>1 x 13 ml</td>
</tr>
<tr>
<td>STOP</td>
<td>STOP solution, white cap, ready to use</td>
<td>1 x 7 ml</td>
</tr>
</tbody>
</table>

3) ADDITIONAL MATERIAL IN THE KIT
- 2 self-adhesive plastic films
- QC protocol
- Instruction sheet
- Protocol sheet

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 and non-reconstituted STDs and CTRL 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 export@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 aluminium bag. Store unused strips with desiccant at 4°C (2-8°C) in the aluminium 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.
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

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

For further information on assay characteristics please visit our website www.bmgrp.com (Validation Data) or contact our customer service by e-mail export@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.

<table>
<thead>
<tr>
<th></th>
<th>Intra-assay (n=5)</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Inter-assay (n=9)</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean (pmol/l)</strong></td>
<td>19.9</td>
<td>80.1</td>
<td></td>
<td>19.7</td>
<td>80.4</td>
<td></td>
</tr>
<tr>
<td><strong>SD (pmol/l)</strong></td>
<td>0.5</td>
<td>2.7</td>
<td></td>
<td>0.6</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td><strong>CV (%)</strong></td>
<td>3</td>
<td>3</td>
<td></td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

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 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.

Avoid contact with skin and mucous membrane. 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 with water immediately after contact!

13) LITERATURE

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.
Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.