Quantikine® ELISA

Human BMP-7 Immunoassay

Catalog Number DBP700
SBP700
PDBP700

For the quantitative determination of human Bone Morphogenetic Protein 7 (BMP-7) concentrations in cell culture supernates, serum, plasma, urine, and bone tissue extracts.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

The bone morphogenetic proteins (BMP) make up a subgroup of the transforming growth factor β (TGF-β) superfamily. As the name implies, BMPs were originally identified as regulators of cartilage and bone formation. They have since been implicated in embryogenesis and morphogenesis of various tissues and organs and can regulate growth, differentiation, chemotaxis, and apoptosis in a variety of cell types (1-5).

The gene for BMP-7, also known as osteogenic protein 1 (OP-1), encodes a 431 amino acid (aa) precursor that contains a 29 aa signal sequence, a 263 aa pro-peptide, and a 139 aa mature protein (6, 7). BMP pro-peptides are removed by proteolysis, enabling mature BMPs to form active disulfide linked homodimers or heterodimers. For example, BMP-7 and BMP-2 form heterodimers that appear to have greater biological activity than the homodimeric forms (8, 9). The dimer can bind and oligomerize a receptor complex that consists of type I and type II receptor serine/threonine kinases that transduce signals via Smad family transcription factors (10). The activities of BMP-7 may be affected by binding endogenous antagonists including noggin, follistatin, cerberus, and gremlin (11). Based on homology, human BMP-5, -6, -7, and -8 make up a subset within the BMP family, sharing approximately 60%-70% aa sequence identity (10). In addition, human and mouse BMP-7 are 98% identical at the aa level.

BMP-7 is developmentally expressed in several human tissue types including olfactory epithelium, intestinal epithelium, perichondria, hypertrophic cartilage, periosteum, telencephalon, spinal cord, and kidney (12). BMP-7 is well known for its putative role as an osteogenic factor *in vivo*, and it has been implicated as a bone-stimulating agent for spinal fusion therapy and the treatment of non-union fractures (13-18). Knockout studies also suggest important roles for BMP-7 in eye development, and BMP-7 deficiency is lethal soon after birth due to disruptions in kidney formation (15, 16, 19). In addition to its important role in development, certain studies suggest that BMP-7 may offer protection from injury in models of renal disease (2, 20, 21). For instance, experimentally elevating the levels of circulating BMP-7 reduces the severity of injury after ischemic acute renal failure (22). BMP-7 also has been used successfully in treating models of adynamic bone disorder (ABD), an ailment characterized by various skeletal abnormalities resulting from chronic kidney disease (22, 23). In the nervous system, putative BMP-7 functions include acting as a neurotrophic factor and regulator of neuronal activity, outgrowth, and differentiation (22, 24-29).

The Quantikine Human BMP-7 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human BMP-7 in cell culture supernates, serum, plasma, urine, and bone tissue extracts. It contains CHO cell-expressed recombinant human BMP-7 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human BMP-7 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human BMP-7.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human BMP-7 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BMP-7 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human BMP-7 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of BMP-7 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

<table>
<tr><th>PART</th><th>PART #</th><th>CATALOG # DBP700</th><th>CATALOG # SBP700</th><th>DESCRIPTION</th><th>STORAGE OF OPENED/ RECONSTITUTED MATERIAL</th></tr>
<tr><td>Human BMP-7 Microplate</td><td>893140</td><td>1 plate</td><td>6 plates</td><td>96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human BMP-7.</td><td>Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Human BMP-7 Conjugate</td><td>893141</td><td>1 vial</td><td>6 vials</td><td>21 mL/vial of a monoclonal antibody specific for human BMP-7 conjugated to horseradish peroxidase with preservatives.</td><td rowspan="8">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Human BMP-7 Standard</td><td>893142</td><td>1 vial</td><td>6 vials</td><td>Recombinant human BMP-7 in a buffered protein solution with preservatives; lyophilized. Refer to the vial label for reconstitution volume.</td></tr>
<tr><td>Assay Diluent RD1-9</td><td>895167</td><td>1 vial</td><td>6 vials</td><td>11 mL/vial of a buffered protein solution with preservatives. May contain a precipitate. Warm to room temperature, and mix gently to dissolve. If the precipitate does not completely dissolve, mix well during use.</td></tr>
<tr><td>Calibrator Diluent RD5P Concentrate</td><td>895151</td><td>1 vial</td><td>6 vials</td><td>21 mL/vial of a concentrated buffered protein solution with preservatives. Use diluted 1:2 for bone extract/serum/plasma samples. Use diluted 1:10 for cell culture supernate/urine samples.</td></tr>
<tr><td>Wash Buffer Concentrate</td><td>895003</td><td>1 vial</td><td>6 vials</td><td>21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative.
May turn yellow over time.</td></tr>
<tr><td>Color Reagent A</td><td>895000</td><td>1 vial</td><td>6 vials</td><td>12 mL/vial of stabilized hydrogen peroxide.</td></tr>
<tr><td>Color Reagent B</td><td>895001</td><td>1 vial</td><td>6 vials</td><td>12 mL/vial of stabilized chromogen (tetramethylbenzidine).</td></tr>
<tr><td>Stop Solution</td><td>895032</td><td>1 vial</td><td>6 vials</td><td>6 mL/vial of 2 N sulfuric acid.</td></tr>
<tr><td>Plate Sealers</td><td>N/A</td><td>4 strips</td><td>24 strips</td><td colspan="2">Adhesive strips.</td></tr>
</table>

* Provided this is within the expiration date of the kit.

DBP700 contains sufficient materials to run an ELISA on one 96 well plate.
SBP700 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDBP700). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human BMP-7 Controls (optional; R&D Systems, Catalog # QC51).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Significant levels of BMP-7 may be found in fetal bovine, bovine, porcine, murine, goat, and rabbit sera. The background level of BMP-7 in control medium should be determined and subtracted from samples of conditioned medium.*

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Hemolyzed samples are not suitable for use in this assay.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Bone Tissue - Extract demineralized bone samples in 4 M Guanidine-HCl and protease inhibitors (30, 31). Dissolve the final sample in 2 M Guanidine-HCl.

Note: *Extracts can also be done in Urea (31, 32).*

SAMPLE PREPARATION

Bone extract samples must be diluted in Calibrator Diluent RD5P (diluted 1:2) prior to assay so that the final concentration of Guanidine-HCl is ≤ 0.5 M or the final concentration of Urea is ≤ 1 M.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

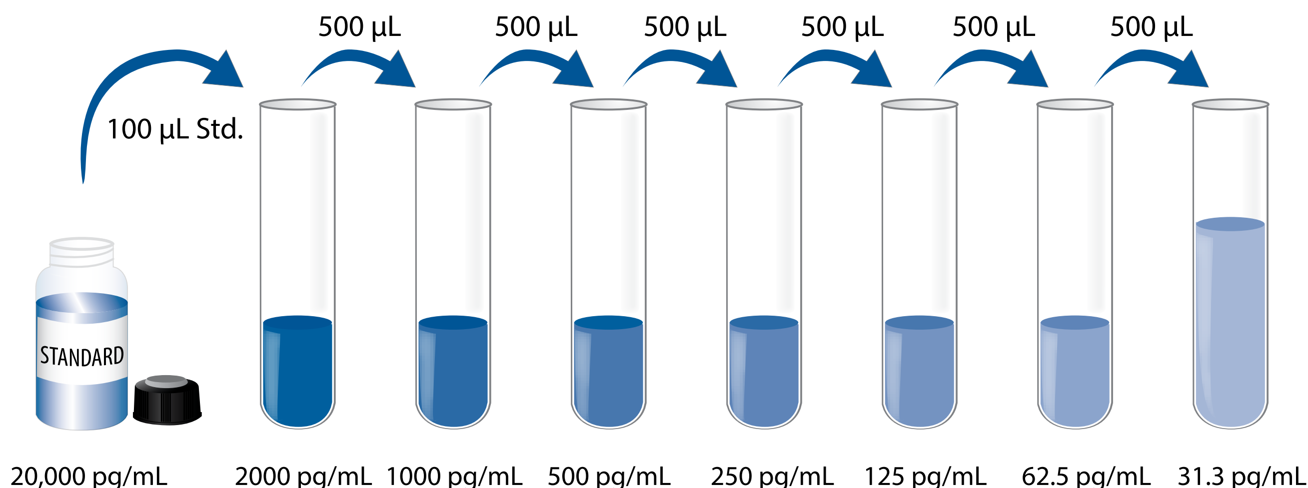
Calibrator Diluent RD5P (diluted 1:2) - **For bone extracts, serum and plasma samples.** Add 5.0 mL of Calibrator Diluent RD5P Concentrate to 5.0 mL of deionized or distilled water to prepare 10 mL of Calibrator Diluent RD5P (diluted 1:2).

Calibrator Diluent RD5P (diluted 1:10) - **For cell culture supernate and urine samples.** Add 1.0 mL of Calibrator Diluent RD5P Concentrate to 9.0 mL of deionized or distilled water to prepare 10 mL of Calibrator Diluent RD5P (diluted 1:10).

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human BMP-7 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human BMP-7 Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:2) (*for bone extracts/serum/plasma samples*) or Calibrator Diluent RD5P (diluted 1:10) (*for cell culture supernate/urine samples*) into the 2000 pg/mL tube. Pipette 500 µL of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of Assay Diluent RD1-9 to each well. *Warm the Assay Diluent to room temperature, and mix well if precipitate is present.*
4. Add 50 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human BMP-7 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Bone samples require extraction and dilution.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

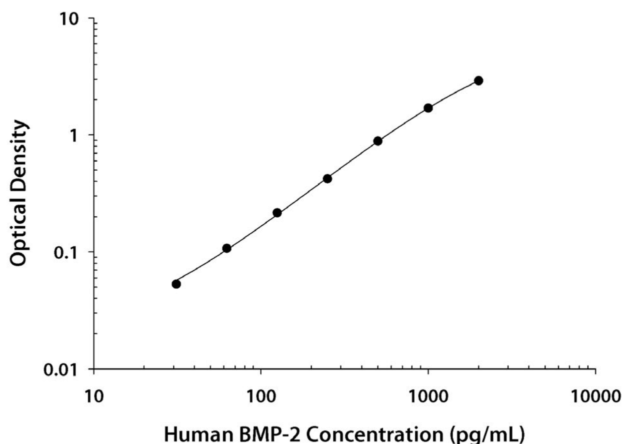
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human BMP-7 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

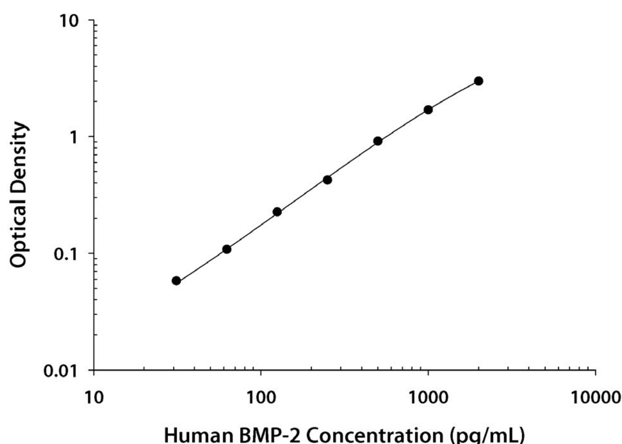
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.024 0.025	0.025	—
31.3	0.075 0.080	0.078	0.053
62.5	0.131 0.133	0.132	0.107
125	0.239 0.243	0.241	0.216
250	0.441 0.450	0.446	0.421
500	0.892 0.926	0.909	0.884
1000	1.698 1.734	1.716	1.691
2000	2.903 2.942	2.923	2.898

SERUM/PLASMA/BONE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.024 0.024	0.024	—
31.3	0.081 0.083	0.082	0.058
62.5	0.128 0.136	0.132	0.108
125	0.243 0.256	0.250	0.226
250	0.445 0.450	0.448	0.424
500	0.917 0.954	0.936	0.912
1000	1.694 1.735	1.715	1.691
2000	2.944 3.063	3.004	2.980

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	370	814	1241	356	752	1148
Standard deviation	20.6	26.8	40.9	27.5	54.5	81.5
CV (%)	5.6	3.3	3.3	7.7	7.2	7.1

SERUM/PLASMA/BONE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	365	783	1194	336	718	1110
Standard deviation	22.1	35.9	80.7	31.9	64.9	86.4
CV (%)	6.1	4.6	6.8	9.5	9.0	7.8

RECOVERY

The recovery of human BMP-7 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	90-114%
Serum (n=4)	100	90-105%
EDTA plasma (n=4)	100	93-105%
Heparin plasma (n=4)	106	96-115%
Urine (n=4)	97	90-104%
Bone extraction solution (n=2)	96	88-102%

SENSITIVITY

Ninety-five assays were evaluated and the minimum detectable dose (MDD) of human BMP-7 ranged from 0.79-7.83 pg/mL. The mean MDD was 2.44 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

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LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human BMP-7 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cellculture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine (n=4)	Bone extraction solution (n=2)
1:2	Average % of Expected	96	102	97	96	93	99
	Range (%)	93-98	99-105	96-99	89-99	87-97	96-102
1:4	Average % of Expected	96	99	100	93	96	102
	Range (%)	92-101	96-101	98-104	88-101	89-99	96-107
1:8	Average % of Expected	100	100	104	97	101	103
	Range (%)	98-102	97-103	99-114	86-108	95-106	98-109
1:16	Average % of Expected	94	99	101	99	103	107
	Range (%)	91-97	96-104	96-110	91-103	100-106	99-115

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant human BMP-7 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Thirty-six matched serum and plasma samples from apparently healthy volunteers were evaluated for the presence of human BMP-7 in this assay. Thirty-five of the matched samples read below the low standard, 31.3 pg/mL. One matched set measured approximately 53 pg/mL. No medical histories were available for the donors used in this study.

Urine - Ten urine samples were evaluated for the presence of human BMP-7 in this assay. All samples measured below the low standard, 31.3 pg/mL.

Cell Culture Supernates:

Human peripheral mononuclear blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of natural human BMP-7. No detectable levels were observed.

U2OS human osteosarcoma cells and MDA-MB-231 human breast cancer cells were also tested for levels of natural human BMP-7. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human BMP-7.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human BMP-7 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Activin A
Activin RIA
Activin RIIA
Activin RIIB
BMP-1.1 (Astacin family)
BMP-2
BMP-3
BMP-3b
BMP-4
BMP-5
BMP-6
BMP-8b
BMP-10
BMP-15
BMPR-1A
BMPR-1B
BMPR-II
Cerberus
COCO/Dante
Follistatin$_{288}$
Follistatin$_{300}$
Follistatin$_{315}$
Inhibin A
Inhibin B
LAP
TGF-α
TGF-β1
TGF-β1.2
TGF-β2
TGF-β3
TGF-β sRI
TGF-β sRII
TGF-β sRIII

Recombinant mouse:

BMP-3b
BMPR-1A
BMPR-1B
Chordin
Gremlin
PRDC
SOST

Other recombinants:

rat Agrin
zebrafish BMP-2
chicken Caronte
porcine TGF-β2
amphibian TGF-β5

Natural proteins:

human TGF-β1
porcine TGF-β1

Recombinant mouse Noggin was found to interfere at concentrations > 12.5 ng/mL.

Recombinant human DAN was found to interfere at concentrations > 25 ng/mL.

The BMP-2/BMP-7 heterodimer cross-reacts approximately 2% in this assay.

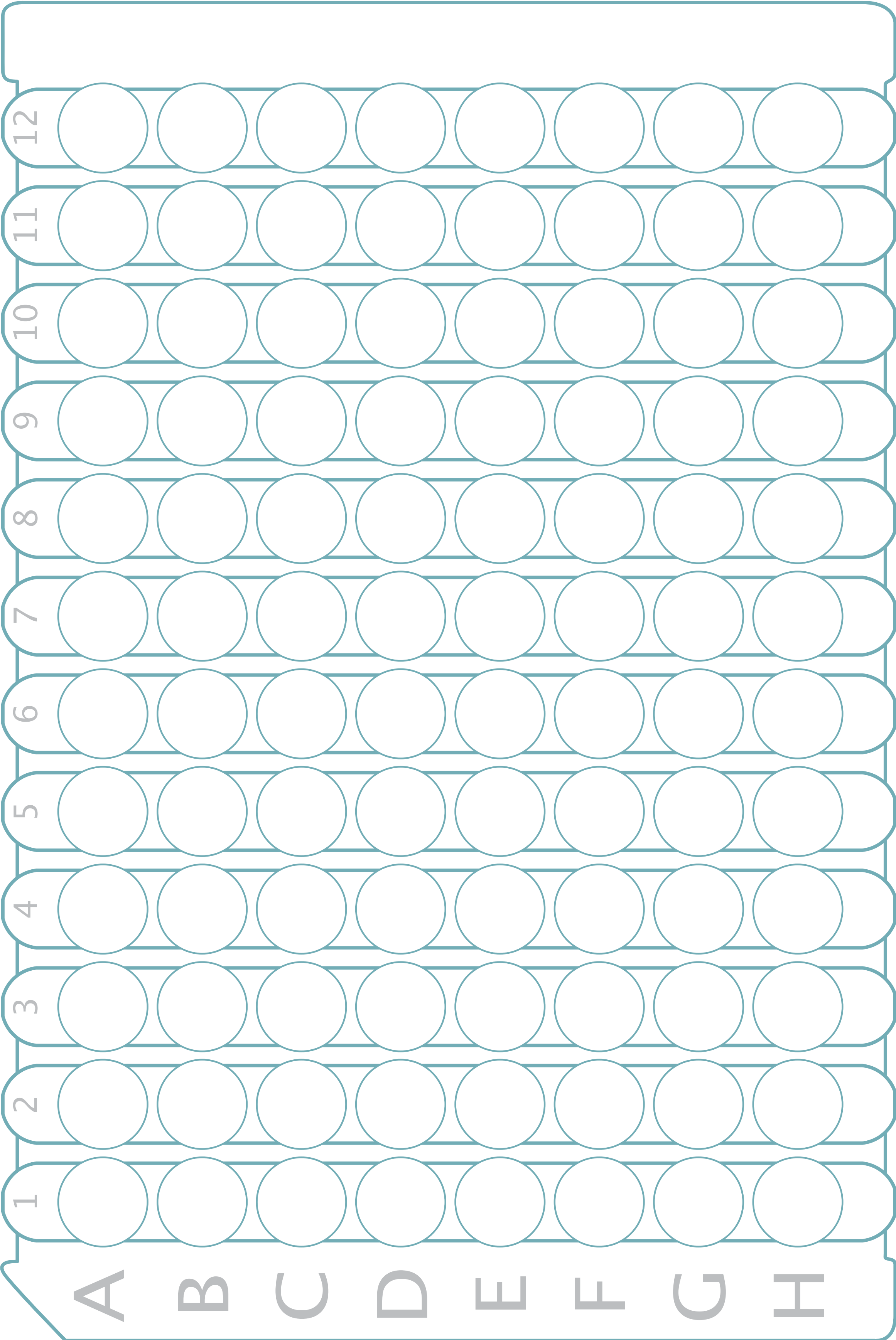
For research use only. Not for use in diagnostic procedures.

REFERENCES

1. Granjeiro, J.M. *et al.* (2005) Braz. J. Med. Biol. Res. **38**:1463.
2. Simic, P. and S. Vukicevic (2005) Cytokine Growth Factor Rev. **16**:299.
3. Canalis, E. *et al.* (2003) Endocr. Rev. **24**:218.
4. Shimasaki, S. *et al.* (2004) Endocr. Rev. **25**:72.
5. Chen, D. *et al.* (2004) Growth Factors **22**:233.
6. Ozkaynak, E. *et al.* (1990) EMBO J. **9**:2085.
7. Celeste, A.J. *et al.* (1990) Proc. Natl. Acad. Sci. USA **87**:9843.
8. Israel, D.I. *et al.* (1996) Growth Factors **13**:291.
9. Zhu, W. *et al.* (2004) J. Bone Miner. Res. **19**:2021.
10. Sebald, W. *et al.* (2004) Biol. Chem. **385**:697.
11. Balemans, W. and W. Van Hul (2002) Dev. Biol. **250**:231.
12. Helder, M.N. *et al.* (1995) J. Histochem. Cytochem. **43**:1035.
13. Hak, D.J. *et al.* (2006) J. Orthop. Res. **24**:11.
14. Dimitriou, R. *et al.* (2005) Injury **36 Suppl 4**:S51.
15. Dudley, A.T. *et al.* (1995) Genes Dev. **9**:2795.
16. Luo, G. *et al.* (1995) Genes Dev. **9**:2808.
17. Bilic, R. *et al.* (2006) Int. Orthop. **30**:128.
18. Kanayama, M. *et al.* (2006) Spine **31**:1067.
19. Karsenty, G. *et al.* (1996) Ann. N.Y. Acad. Sci. **785**:98.
20. Zeisberg, M. (2006) Nephrol. Dial. Transplant. **21**:568.
21. Zeisberg, M. *et al.* (2003) Nat. Med. **9**:964.
22. Vukicevic, S. *et al.* (1998) J. Clin. Invest. **102**:202.
23. Lund, R.J. *et al.* (2004) J. Am. Soc. Nephrol. **15**:359.
24. Zuch, C.L. *et al.* (2004) Brain Res. **1010**:10.
25. Dale, J.K. *et al.* (1997) Cell **90**:257.
26. Shen, W. *et al.* (2004) Eur. J. Neurosci. **20**:2031.
27. Yabe, T. *et al.* (2002) J. Neurosci. Res. **68**:161.
28. Augsburger, A. *et al.* (1999) Neuron **24**:127.
29. Lein, P. *et al.* (1995) Neuron **15**:597.
30. Takaoka, K. *et al.* (1980) Clin. Orthop. Relat. Res.**148**:274.
31. Sampath, T.K. and A.H. Reddi (1981) Proc. Natl. Acad. Sci. USA **78**(12):7599.
32. Urist, M.R. *et al.* (1982) Clin. Orthop. Relat. Res. **162**:219.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



For research use only. Not for use in diagnostic procedures.

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