# Quantikine®

## Human BMP-7 Immunoassay

Catalog Number DBP700

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

***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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# 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 bone tissue extracts, cell culture supernates, serum, plasma, and urine. 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 the Quantikine Human BMP-7 kit can be used to determine relative mass values for naturally occurring BMP-7.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for 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 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.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

## MATERIALS PROVIDED

**BMP-7 Microplate** (Part 893140) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against BMP-7.

**BMP-7 Conjugate** (Part 893141) - 21 mL of monoclonal antibody against BMP-7 conjugated to horseradish peroxidase with preservatives.

**BMP-7 Standard** (Part 893142) - 20 ng of recombinant human BMP-7 in a buffered protein solution with preservatives, lyophilized.

**Assay Diluent RD1-9** (Part 895167) - 11 mL of a buffered protein solution with preservatives. Assay Diluent RD1-9 may contain a precipitate. Warm to room temperature, and mix gently to dissolve. If the precipitate does not completely dissolve, mix well during use.

**Calibrator Diluent RD5P Concentrate** (Part 895151) - 21 mL of a concentrated buffered protein solution with preservatives.

**Wash Buffer Concentrate** (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.

**Color Reagent A** (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

**Color Reagent B** (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

**Stop Solution** (Part 895032) - 6 mL of 2 N sulfuric acid.

**Plate Covers** - 4 adhesive strips.

## STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 - 8° C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/ Reconstituted Reagents</td><td>Diluted Wash Buffer</td><td rowspan="8">May be stored for up to 1 month at 2 - 8° C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Assay Diluent RD1-9</td></tr>
<tr><td>Calibrator Diluent RD5P</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*</td></tr>
</table>

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

## 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.
- Human BMP-7 Controls (optional; available from R&D Systems).
- **Polypropylene tubes.**

## PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

## SAMPLE COLLECTION AND STORAGE

**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.*

**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).*

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes 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 heparin or EDTA 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, assay immediately or aliquot and store at ≤ -20° C. Avoid repeated freeze-thaw cycles. For more information on collecting and handling urine specimens, refer to the National Committee for Clinical Laboratory Standards (NCCLS) publication GP16-T.

## SAMPLE PREPARATION

Bone extract samples must be diluted in Calibrator Diluent RD5P (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. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

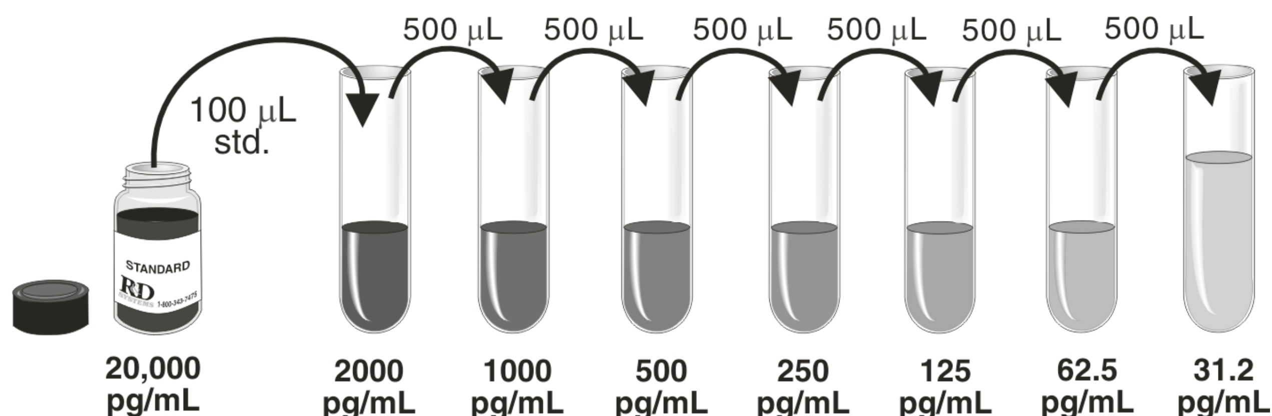
**Calibrator Diluent RD5P (1:2)** - *(For bone extracts, serum and plasma samples)* Add 5.0 mL of Calibrator Diluent RD5P Concentrate to 5.0 mL of deionized or distilled water.

**Calibrator Diluent RD5P (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.

**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.

**BMP-7 Standard** - Reconstitute the BMP-7 Standard with 1.0 mL of 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 (1:2) *(for bone extracts, serum and plasma samples)* or Calibrator Diluent RD5P (1:10) *(for cell culture supernate and urine samples)* into the 2000 pg/mL tube. Pipette 500 μL of the appropriate Calibrator Diluent into the remaining tube. 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

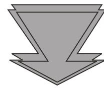
**Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.**

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, 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 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.

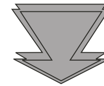
*Bone samples require extraction and dilution.

## ASSAY PROCEDURE SUMMARY

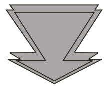
**1.** Prepare reagents, samples, and standards as instructed.



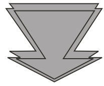
**2.** Add 100 µL Assay Diluent RD1-9 to each well.



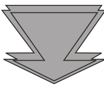
**3.** Add 50 µL Standard, control, or sample* to each well. Incubate 2 hrs. on the shaker at RT.



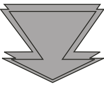
**4.** Aspirate and wash 4 times.



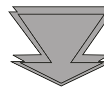
**5.** Add 200 µL Conjugate to each well. Incubate 2 hrs. on the shaker at RT.



**6.** Aspirate and wash 4 times.



**7.** Add 200 µL Substrate Solution to each well. Incubate 30 min. **on the benchtop. Protect from light.**



**8.** Add 50 µL Stop Solution to each well. Read at 450 nm within 30 min. λ correction 540 or 570 nm

*Bone samples require extraction and dilution.

## CALCULATION OF RESULTS

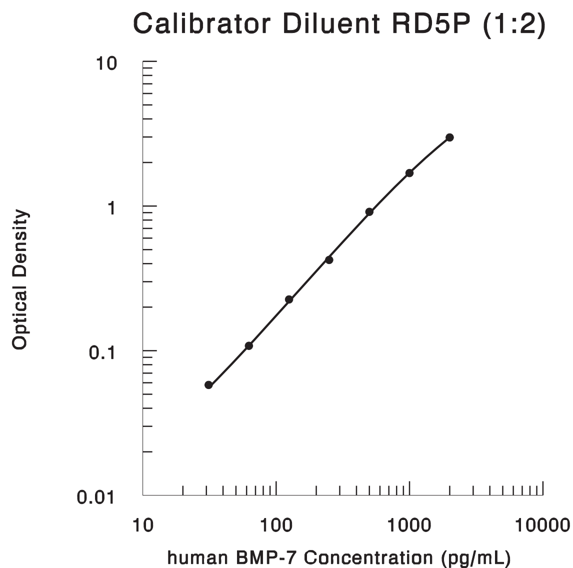
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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 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.

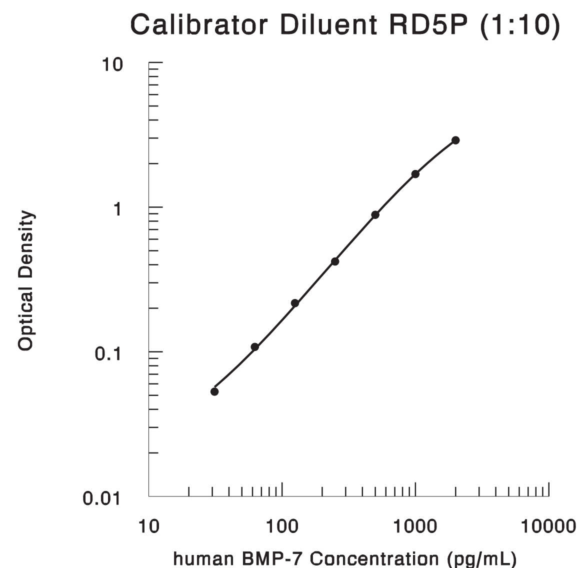
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL DATA

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




| pg/mL | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.024<br>0.024 | 0.024 | — |
| 31.2 | 0.081<br>0.083 | 0.082 | 0.058 |
| 62.5 | 0.128<br>0.136 | 0.132 | 0.108 |
| 125 | 0.243<br>0.256 | 0.250 | 0.226 |
| 250 | 0.445<br>0.450 | 0.448 | 0.424 |
| 500 | 0.917<br>0.954 | 0.936 | 0.912 |
| 1000 | 1.694<br>1.735 | 1.715 | 1.691 |
| 2000 | 2.944<br>3.063 | 3.004 | 2.980 |




| pg/mL | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.024<br>0.025 | 0.025 | — |
| 31.2 | 0.075<br>0.080 | 0.078 | 0.053 |
| 62.5 | 0.131<br>0.133 | 0.132 | 0.107 |
| 125 | 0.239<br>0.243 | 0.241 | 0.216 |
| 250 | 0.441<br>0.450 | 0.446 | 0.421 |
| 500 | 0.892<br>0.926 | 0.909 | 0.884 |
| 1000 | 1.698<br>1.734 | 1.716 | 1.691 |
| 2000 | 2.903<br>2.942 | 2.923 | 2.898 |

## 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.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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.

## 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.

**Bone/Serum/Plasma 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 |

**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 |

## RECOVERY

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

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

## LINEARITY

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

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

## SENSITIVITY

Ninety-five assays were evaluated and the minimum detectable dose (MDD) of 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.

## 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 drawn from apparently healthy volunteers were evaluated for the presence of BMP-7 in this assay. No medical histories were available for the donors used in this study. Thirty-five of the matched samples read below the low standard, 31.2 pg/mL. One matched set measured approximately 53 pg/mL.

**Urine** - Ten urine samples were evaluated for the presence of BMP-7 in this assay. All samples measured below the low standard, 31.2 pg/mL.

**Cell Culture Supernates** -
Human peripheral mononuclear blood cells ($1 \times 10^6$ 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 supernate were removed and assayed for levels of natural BMP-7. No detectable levels were observed.

U2-OS cells and MDA-MB-231 cells were also tested for levels of natural BMP-7. No detectable levels were observed.

# SPECIFICITY

This assay recognizes recombinant and natural 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-$\alpha$
TGF-$\beta$1
TGF-$\beta$1.2
TGF-$\beta$2
TGF-$\beta$3
TGF-$\beta$ sRI
TGF-$\beta$ sRII
TGF-$\beta$ sRIII

**Recombinant mouse:**
BMP-3b
BMPR-1A
BMPR-1B
Chordin
Gremlin
PRDC
SOST

**Recombinant rat:**
Agrin

**Recombinant amphibian:**
TGF-$\beta$5

**Recombinant chicken:**
Caronte

**Recombinant porcine:**
TGF-$\beta$2

**Recombinant zebrafish:**
BMP-2

**Other proteins:**
human TGF-$\beta$1
porcine TGF-$\beta$1

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.

## REFERENCES


1. Granjeiro, J.M. *et al.* (2005) Braz. J. Med. Biol. Res. **38**:1463.
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## PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.



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