

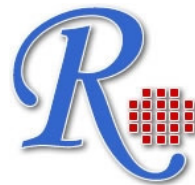
RayBio®

Human BMP-4 ELISA Kit

**User Manual (for Cell Lysate and
Tissue Lysate)
(Revised Mar 1, 2012)**

**RayBio® Human BMP-4
ELISA Kit Protocol**

(Cat#: ELH-BMP4-001C)



RayBiotech, Inc.

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Protein Array System And Service**

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ELISA Kit Protocol**

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I. INTRODUCTION

The RayBio® Human BMP-4 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of human BMP-4 cell lysate and tissue lysate. This assay employs an antibody specific for human BMP-4 coated on a 96-well plate. Standards and samples are pipetted into the wells and BMP-4 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human BMP-4 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of BMP-4 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

II. REAGENTS

1. BMP-4 Microplate (Item A): 96 wells (12 strips x 8 wells) coated with anti-human BMP-4.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml of 20x concentrated solution.
3. Standards (Item C): 2 vials, recombinant human BMP-4.
4. Sample Diluent Buffer (Item D): 10 ml of 5x concentrated buffer. For Standard/Sample (cell lysate/tissue lysate) diluent.
5. Assay Diluent (Item E): 15 ml of 5x concentrated buffer. For Detection Antibody (Item F) and HRP-Streptavidin concentrate (Item G) diluent.
6. Detection Antibody BMP-4 (Item F): 2 vials of biotinylated anti-human BMP-4 (each vial is enough to assay half microplate).
7. HRP-Streptavidin concentrate (Item G): 200 µl 80x concentrated HRP-conjugated streptavidin.
8. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffered solution.

9. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.
10. Cell lysate buffer (Item J): 5 ml 2x cell lysate buffer.

III. STORAGE

May be stored for up to 6 months at 2° to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution. Opened Microplate Wells or reagents may be store for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

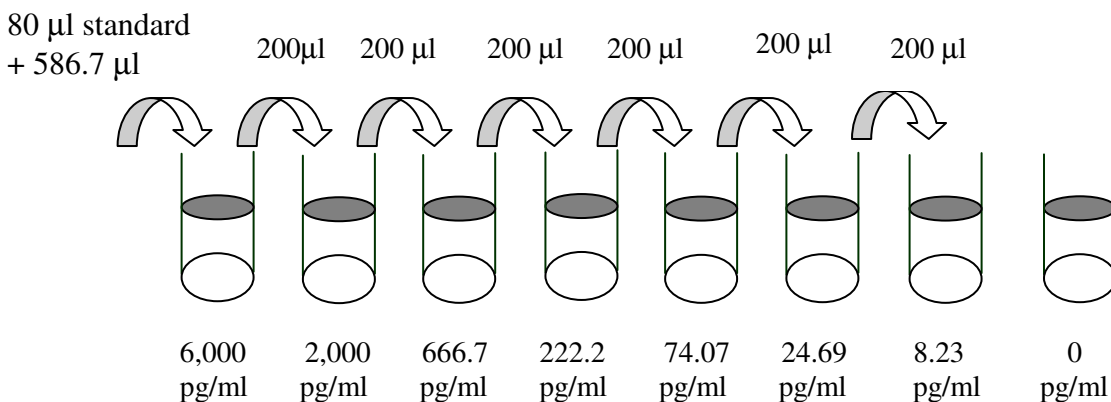
IV. ADDITIONAL MATERIALS REQUIRED

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- 2 Precision pipettes to deliver 2 µl to 1 ml volumes.
- 3 Adjustable 1-25 ml pipettes for reagent preparation.
- 4 100 ml and 1 liter graduated cylinders.
- 5 Absorbent paper.
- 6 Distilled or deionized water.
- 7 Log-log graph paper or computer and software for ELISA data analysis.
- 8 Tubes to prepare standard or sample dilutions.

V. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Sample dilution: Tissue lysate and cell lysate sample should be diluted at least 5-folds with 1x Sample Diluent Buffer.

- Sample Diluent Buffer (Item D) and Assay Diluent (Item E) should be diluted 5-folds with deionized or distilled water before use.
- Preparation of standard: **Briefly spin the vial of Item C.** Add 400 μl 1x Sample Diluent Buffer (Item D) into Item C vial to prepare a 50 ng/ml standard. **Dissolve the powder thoroughly by a gentle mix.** Add 80 μl BMP-4 standard from the vial of Item C, into a tube with 586.7 μl Sample Diluent Buffer to prepare a 6,000 pg/ml stock standard solution. Pipette 400 μl 1x Sample Diluent Buffer into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1x Sample Diluent Buffer serves as the zero standard (0 pg/ml).



- If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
- Briefly spin the Detection Antibody vial (Item F) before use. Add 100 μl of 1x Assay Diuent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 65-foldss with 1x Assay Diuent and used in step 4 of Part VI Assay Procedure.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) before use. HRP-Streptavidin concentrate should be diluted 80-fold with 1x Assay Diluent.

For example: Briefly spin the vial (Item G) and pipette up and down to mix gently . Add 125 μ l of HRP-Streptavidin concentrate into a tube with 10 ml 1x Assay Diluent to prepare a final 80 fold diluted HRP-Streptavidin solution. Mix well.




8. Cell lysate buffer (Item J) should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

VI. ASSAY PROCEDURE:

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 μ l of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well with plate holder and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 μ l) using a multi-channel Pipette 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.

4. Add 100 μ l of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 μ l of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 μ l of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 μ l of Stop Solution (Item I) to each well. Read at 450 nm immediately.

VII. ASSAY PROCEDURE SUMMARY

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

2. Add 100 μ l standard or sample to each well.
Incubate 2.5 hours at room temperature or over night at 4°C.

3. Add 100 μ l prepared biotin antibody to each well.
Incubate 1 hour at room temperature.


4. Add 100 μ l prepared Streptavidin solution.
Incubate 45 minutes at room temperature.



5. Add 100 μ l TMB One-Step Substrate Reagent to each well.
Incubate 30 minutes at room temperature.



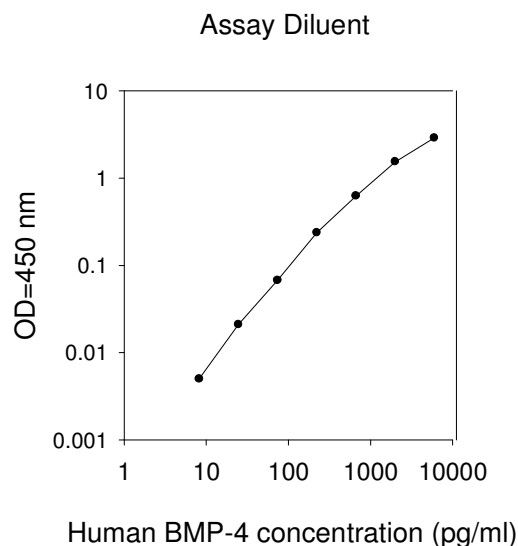
6. Add 50 μ l Stop Solution to each well.
Read at 450 nm immediately.

VIII. CALCULATION OF RESULTS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

A. TYPICAL DATA

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. SENSITIVITY

The minimum detectable dose of BMP-4 is typically less than 15 pg/ml.

C. RECOVERY

Recovery was determined by spiking various levels of human BMP-4 into human tissue lysate and cell lysate. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Tissue lysate	89.47	78-102
Cell lysate	92.45	80-103

D. LINEARITY

Sample Type	Tissue Lysate	Cell lysate
1:2 Average % of Expected Range (%)	84.31 77-103	85.75 77-102
1:4 Average % of Expected Range (%)	86.78 79-102	88.54 80-104

E. REPRODUCIBILITY

Intra-Assay: CV<10%

Inter-Assay: CV<12%

IX. SPECIFICITY

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (*e.g.*, human Angiogenin, BDNF, BLC, BMP-6, BMP-7, ENA-78, FGF-4, IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12 p70, IL-12 p40, IL-13, IL-15, IL-309, IP-10, G-CSF, GM-CSF, IFN- γ , Leptin, MCP-1, MCP-2, MCP-3, MDC, MIP-1 α , MIP-1 β , MIP-1 δ , PARC, PDGF, RANTES, SCF, TARC, TGF- β , TIMP-1, TIMP-2, TNF- α , TNF- β , TPO, VEGF).

X. REFERENCES:

1. Aldinger G et al. Bone morphogenetic protein: a review. *International Orthop.* 15: 169-77 (1991).
2. Bhatia M et al. Bone Morphogenetic Proteins Regulate the Developmental Program of Human Hematopoietic Stem Cells. *Journal of Experimental Medicine* 189(7): 1139-1148 (1999).
3. Wozney JM. The bone morphogenetic protein family and osteogenesis. *Mol. Reprod. Dev.* 32: 160-7 (1992).

XI. TROUBLESHOOTING GUIDE

Problem	Cause	Solution
1. Poor standard curve	<ol style="list-style-type: none"> 1. Inaccurate pipetting 2. Improper standard dilution 	<ol style="list-style-type: none"> 1. Check pipettes 2. Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	<ol style="list-style-type: none"> 1. Too brief incubation times 2. Inadequate reagent volumes or improper dilution 	<ol style="list-style-type: none"> 1. Ensure sufficient incubation time; assay procedure step 2 change to over night 2. Check pipettes and ensure correct preparation
3. Large CV	<ol style="list-style-type: none"> 1. Inaccurate pipetting 	<ol style="list-style-type: none"> 1. Check pipettes
4. High background	<ol style="list-style-type: none"> 1. Plate is insufficiently washed 2. Contaminated wash buffer 	<ol style="list-style-type: none"> 1. Review the manual for proper wash. If using an a plate washer, check that all ports are unobstructed. 2. Make fresh wash buffer
5. Low sensitivity	<ol style="list-style-type: none"> 1. Improper storage of the ELISA kit 2. Stop solution 	<ol style="list-style-type: none"> 1. Store your standard at $<-20^{\circ}\text{C}$ after reconstitution, others at 4°C. Keep substrate solution protected from light 2. Stop solution should be added to each well before measure

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Notes:

This product is for research use only.



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