Human BMP-7 ELISA Kit

Product description

Human Bone Morphogenetic Protein-7 (Human BMP-7) is a member of the TGF-β superfamily and is considered one of the most potent synthetic factors for chondrocytes. The growth factor domain of human BMP-7 shares 98% amino acid sequence identity with mouse and rat BMP-7. BMP-7 acts in various organ systems. It promotes new bone formation and the development of renal units, inhibits differentiation of prostate epithelium, and antagonizes epithelial-mesenchymal transition. Bone Morphogenetic Protein (BMP) signaling regulates a series of cellular processes and plays an important role in early embryonic patterning. It is known that BMP-7 expression has a protective effect on renal injury during diabetic nephropathy and disappears in the early stages of diabetic nephropathy progression. Potential clinical applications of BMP-7 include its neuroprotective effects in stroke animal models.

The Arcegen Human BMP-7 ELISA (Enzyme-Linked Immunosorbent Assay) Kit is an in vitro enzyme-linked immunosorbent assay kit used for quantitatively measuring BMP-7 in serum, plasma, and cell culture supernatants. Specific anti-BMP-7 antibodies are precoated onto high-affinity enzyme plates. Standard samples and test samples are added to the enzyme plate wells and incubated. BMP-7 in the samples binds to the solid-phase antibodies. After washing away unbound substances, detection antibodies are added and incubated, followed by washing and addition of enzyme conjugate (Streptavidin-HRP) for incubation. After washing, TMB substrate solution is added for color development in the dark. The intensity of the color reaction is directly proportional to the concentration of BMP-7 in the sample. The reaction is terminated by adding a stop solution, and the absorbance is measured at a wavelength of 450 nm (with a reference wavelength of 570 - 630 nm).

Specifications

Item Number	P162014S / P162014E
Specification	48 T / 96 T
Detection Range	62.5-4000 pg/mL
Detection Method	Sandwich ELISA
Detection Time	4.5 hours
Sensitivity	44.6 pg/mL
Dilution Linearity	86 - 127%
Recovery Rate	81 - 126%
Intra-assay Variability	3.3%
Inter-assay Variability	4.2%

Components

Component Number	Component Name	Storage Temperature	P162014S	P162014E
P162014-A	ELISA Plate	2~8°C	48 T	96 T
P162014-B	Standard	2~8°C	1 tube	2 tubes
P162014-C	Detection Antibody	2~8°C	60 µL	120 µL
P162014-D	Enzyme Conjugate	2~8°C(Avoid Light)	30 µL	60 µL
P162014-E	Sample Dilution Buffer	2~8°C	8 mL	15 mL
P162014-F	Antibody/Enzyme Dilution Buffer	2~8°C	15 mL	30 mL
P162014-G	20x Wash Buffer	2~8°C	25 mL	50 mL
P162014-H	Substrate Solution	2~8°C(Avoid Light)	8 mL	15 mL
P162014-I	Stop Solution	Room Temperature	5 mL	10 mL
P162014-J	Plate Sealant Film	Room Temperature	3 pieces	5 pieces

Storage

The kit can be stored at 2~8°C or according to the storage conditions provided in the component information to avoid contamination and repeated freeze-thaw cycles. Diluted working concentration reagents should be used immediately and discarded, and they should not be reused. The shelf life is 1 year.

Table 1.Reagent Storage Table After First Use

Component Name	Storage Conditions
ELISA Plate	Unused strips can be returned to the aluminum foil bag,tightly sealed,and stored at 2-8°C to avoid moisture absorption.
Standard	Use within 48 hours after dissolution,store at 2-8°C to avoid contamination.
Detection Antibody	Use within 48 hours after dilution,store at 2~8°C to avoid contamination.
Enzyme Conjugate	
Sample Dilution Buffer	Store at 2~8°C for 1 month,avoiding contamination.
Antibody/Enzyme Dilution Buffer	
20x Wash Buffer	
Substrate Solution	Store at 2~8°C for 1 month,avoiding light exposure.
Stop Solution	Can be stored at room temperature.
Plate Sealant Film	

Instructions

1. Used for quantitative detection of BMP-7 content in serum, plasma, and cell culture supernatant.
2. Please read the instruction manual carefully before using this product.

Notes

1. This product is for research use only.
2. For your safety and health, wear laboratory clothing and disposable gloves when operating.
3. The reagent kit should be used within the shelf life.It is prohibited to mix reagents from different batches.
4. This product can only be used to detect target antigens and samples as indicated in the instruction manual.Other applications require validation by the user, and the reliability and accuracy of the use should be evaluated based on the results.
5. Do not mix or substitute reagents or materials from different batches or suppliers of other kits.

Common Technical Tips

1. When the sample OD value is higher than the S1 OD value, further dilution should be performed in an appropriate sample diluent.
2. Avoid generating foam during mixing.
3. Replace tips promptly when adding standard samples, samples, and others to avoid cross-contamination.
4. Ensure proper sealing of the ELISA plate or complete coverage with plate seal film during incubation.
5. Please completely remove all solutions and buffers during the washing steps.
6. Before dissolving the standard samples, do not invert the standard sample tube randomly. After inverting the standard sample tube, thoroughly mix it up and down after adding the buffer, then centrifuge at low speed.
7. During the experiment, place the reagents according to the instructions.
8. Promptly discard buffers after the experiment; use immediately.
9. Different products have different components in the reagent kit and cannot be used interchangeably.

Other Preparation Materials

1. ELISA reader, measure absorbance at 450 nm (reference wavelength 630 nm).
2. Incubator, automated microplate washer.
3. Pipettes, 1 μL to 1 mL pipettes with corresponding tips.

4. 100 mL and 1 L graduated cylinders.
5. Standard or sample dilution tubes.
6. Blotting paper.
7. Distilled water or deionized water.
8. Computer and analysis software.

Pre-Experiment Preparation

1. Sample Collection and Processing

1) Cell culture supernatant: Centrifuge at 1, 000×g for 10 minutes to remove precipitates. Immediate detection or aliquot and store at -20°C or below.

2) Serum samples: Collect serum using tubes free of pyrogens and endotoxins. Allow blood samples to clot for 30 minutes, then centrifuge at 1, 000×g for 10 minutes. Immediately detect or aliquot and store at -20°C or below.

3) Plasma samples: Collect plasma samples using EDTA, sodium citrate, or heparin anticoagulants. Centrifuge at 1, 000×g for 30 minutes to collect samples. Immediate detection or aliquot and store at -20°C or below.

This assay kit may be applicable to other biological samples. Serum, plasma, and cell culture supernatant have been validated.

[Note] Prior to testing, visible precipitates in samples must be removed. Do not use samples with severe hemolysis or high lipid content. Samples should be aliquoted and stored at -20°C to avoid loss of BMP-7 activity. If testing within 24 hours, samples can be stored at 2~8°C to avoid repeated freeze-thaw cycles. Frozen samples should be slowly brought to room temperature (25°C ± 3°C) and gently mixed before testing.

If sample dilution is necessary, use the specified sample buffer for dilution.

Recommended dilution for normal serum/plasma samples (for reference only): 1:1 dilution with sample diluent. Recommended for cell culture supernatant (for reference only): Undiluted.

Due to variations in the target protein content of samples, the dilution ratio for each sample is recommended based on pre-experiment results or determined according to actual conditions.

2. Preparation of the ELISA Plate

The ELISA plate should be brought to room temperature before use.Unused strips should be promptly sealed in a desiccant and stored at 2~8°C, with each sample requiring multiple wells for experimentation.

3. Reagent Preparation

Before use, all reagents and samples need to be equilibrated to room temperature. To ensure experimental accuracy, please complete the preparation within 15 minutes before use.

1) 1×Wash Buffer Preparation: Equilibrate the concentrated solution to room temperature, dissolve completely without crystallization. Mix well, take 25 mL of 20×Wash Buffer into distilled water, then bring to a final volume of 500 mL; the specific volume can be prepared according to the amount used each time.

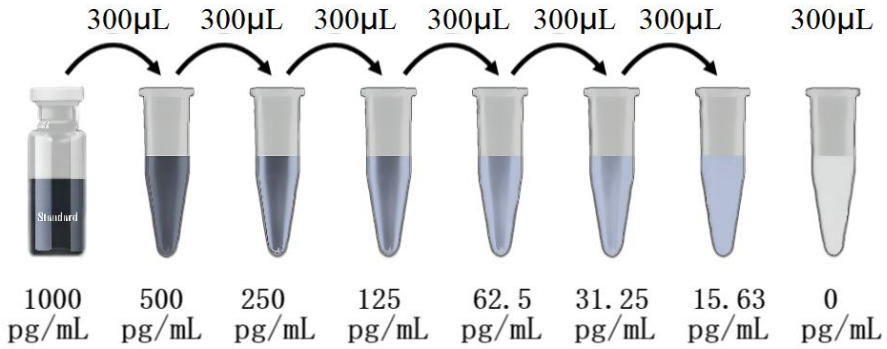
2) Detection Antibody Preparation: Before use, centrifuge at 10, 000 rpm for 20 seconds, then dilute with antibody diluent at a 1:100 ratio to working concentration, for example, take 60 µL and bring to a final volume of 6 mL antibody diluent; the specific volume can be prepared according to the amount used each time, mix well.

3) Enzyme Conjugate Preparation: Before use, centrifuge at 10, 000 rpm for 20 seconds, then dilute with enzyme diluent at a 1:200 ratio to working concentration, for example, pipette 30 µL and dilute with antibody/enzyme diluent to 6 mL; the specific volume can be prepared according to the amount used each time, mix well.

4) Preparation of Standard Curve: Prepare 7 sterile 1.5 mL centrifuge tubes, label them according to the standard curve concentration. Prepare S1: Take one vial of standard lyophilized product and dissolve it in sample diluent according to the labeled amount, mix well, marking it as 4000 pg/mL. Add 300 µL of 1× sample diluent to each centrifuge tube, first take 300 µL of S1, add it to the first centrifuge tube, mix well, then take 300 µL to the next labeled concentration centrifuge tube, mix well, prepare a 2-fold gradient dilution standard curve, starting with the highest concentration marked as 4000 pg/mL, and the lowest concentration as 62.5 pg/mL, follow the preparation method below. Prepare the standard curve for each experiment, different assay kits, and standard curves at different times should not be mixed. When testing samples, 100 µL of standard solution is required for each well, ensure that the preparation volume is higher than the required volume to avoid insufficient usage.

Table 2. BMP-7 Standard Curve Preparation (62.5 - 4000 pg/mL)

Standard Curve	Diluent(µL)	Volume of Standard Added(µL)	Final Concentration of Standard (pg/mL)
S1	As labeled	/	4000
S2	300	300	2000
S3	300	300	1000
S4	300	300	500
S5	300	300	250
S6	300	300	125
S7	300	300	62.5
Blank	300	0	0



Operating Instructions

Before use, all reagents and test samples need to be equilibrated to room temperature. It is strongly recommended to perform duplicate measurements for all standard samples and test samples.

1. Reagent preparation: Prepare various test reagents, diluted standard samples, and test samples.
2. Determination of enzyme plate strips: Calculate the required enzyme plate strips for test samples and standard samples. Remove the enzyme plate strips from the aluminum foil bag, return the remaining enzyme plate strips to the aluminum foil bag, seal the bag tightly, and store at low temperature.
3. Soaking of enzyme plate: Add 1× wash solution (350 μL/well) to soak the enzyme plate. After standing for 30 seconds, discard the liquid from the wells and pat dry the enzyme plate. The liquid volume has a significant impact on the test results, ensure that there is no residual wash solution after the final patting of the plate.
4. Sample incubation: Add various gradient standard samples and diluted test samples, 100 μL/well. Ensure that the spotting is completed within 15 minutes and incubate at room temperature for 2 hours.
5. Washing of enzyme plate: Discard the liquid from the wells, add 1× wash solution (350 μL/well) to wash the plate 5 times, and pat dry the enzyme plate.
6. Incubation with detection antibody: Add the detection antibody prepared to working concentration to the enzyme plate, 100 μL/well. Incubate at room temperature for 2 hours.
7. Washing of enzyme plate: Discard the liquid from the wells, add 1× wash solution (350 μL/well) to wash the plate 5 times, and pat dry the enzyme plate.
8. Incubation with enzyme conjugate: Add the enzyme conjugate prepared to working concentration to the enzyme plate, 100 μL/well. Incubate at room temperature for 20 minutes.
9. Washing of enzyme plate: Discard the liquid from the wells, add 1× wash solution (350 μL/well) to wash the plate 5 times, and pat dry the enzyme plate.
10. Color development: Equilibrate the substrate solution to room temperature for 10 minutes before use. Add the substrate solution to the enzyme plate, 100 μL/well. Incubate at room temperature in the dark for 15 minutes.

11. Termination: Add 50 µL/well stop solution to the enzyme plate. At this point, the color changes from blue to yellow. Gently shake the enzyme plate to ensure uniform color development.
12. Reading: Read the absorbance values at 450 nm/630 nm within 10 minutes.

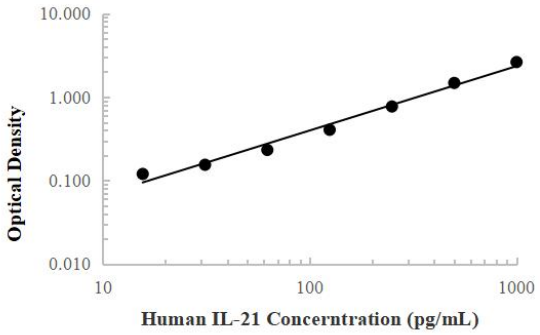
Standard Curve Establishment

Calculate the average OD value of duplicate wells for both standard samples and test samples, and subtract the average OD value of blank wells to obtain the calibrated OD value. Plot the standard curve with the logarithm of the standard sample concentration as the x-axis and the logarithm of the calibrated OD value as the y-axis. Various plotting and statistical software can be used to assist in drawing the standard curve and calculating the concentration of unknown samples. The four-parameter fitting method often yields better fitting results, while other methods such as linear fitting may also yield satisfactory results, which need to be analyzed based on specific experimental data.

Experimental Data

1. Standard Curve Data

Fitting data to generate a standard curve for analysis of experimental data.



Standard Curve Graph

Concentration(pg/mL)	Absorbance		Mean Value	Calibration Value
4000	2.366	2.458	2.412	2.411
2000	1.527	1.554	1.541	1.546
1000	0.844	0.913	0.879	0.869
500	0.427	0.455	0.441	0.451
250	0.226	0.232	0.229	0.229
125	0.127	0.129	0.128	0.121
62.5	0.068	0.065	0.067	0.070
0	0.016	0.016	0.016	/

2. Sensitivity Detection

The minimum detection limit for BMP-7 is 44.6 pg/mL, calculated by determining the mean and standard deviation of the OD values from 20 repeated measurements of the blank wells.

3. Precision Detection

Intra-assay Precision

The precision within the enzyme-linked immunosorbent assay (ELISA) plate was assessed by performing 10 repeated measurements of 3 samples with known concentrations, aiming to evaluate the intra-assay precision.

Inter-assay Precision

The precision between enzyme-linked immunosorbent assay (ELISA) plates was evaluated by conducting 30 repeated measurements of 3 samples with known concentrations to assess the inter-assay precision.

Project	Intra-assay Precision			Inter-asaay Precison		
Sample	1	2	3	1	2	3
	10	10	10	30	30	30
Mean Value	2004.8	458.1	115.7	1999.2	453.6	114.2
Standard Deviation	106.5	12.6	2.0	101.1	17.1	4.1
Coefficient of Variation (%)	5.3	2.7	1.8	5.1	3.8	3.6

4. Recovery Rate Detection

Recovery rates were determined by adding different levels of BMP-7 to the samples. The recovery rates are as follows:

Sample Types	Average Recovery Rate (%)	Range (%)
Serum	101.3	86.1-126.4
Plasma	103.9	81.8-122.1
Cell culture supernatant	103.6	89.0-126.4

5. Dilution Linearity Test

Serum Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	113.9%	105.8-127.8
1:04	107.7%	104.1-113.9
1:08	97.4%	90.7-101.4
1:16	95.3%	87.2-101.6

Plasma Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	110.8%	106.8-113.5
1:04	113.9%	95.8-122.2
1:08	96.5%	86.3-106.6
1:16	103.9%	91.5-118.6

Cell Culture Supernatant Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	102.0%	101-103.1
1:04	110.9%	104.3-119.7
1:08	97.8%	94.3-102.0
1:16	98.6%	94.9-103.3

6. Sample Values

The assay kit was applied to test samples from several healthy volunteers, whose medication history was not provided.

Sample Types	Sample Number	Mean Value (pg/mL)	Sample Value(pg/mL)
Serum	6	n.d.	n.d.
Plasma	12	n.d.	n.d.
Cell Culture Supernatant	8	n.d.	n.d.

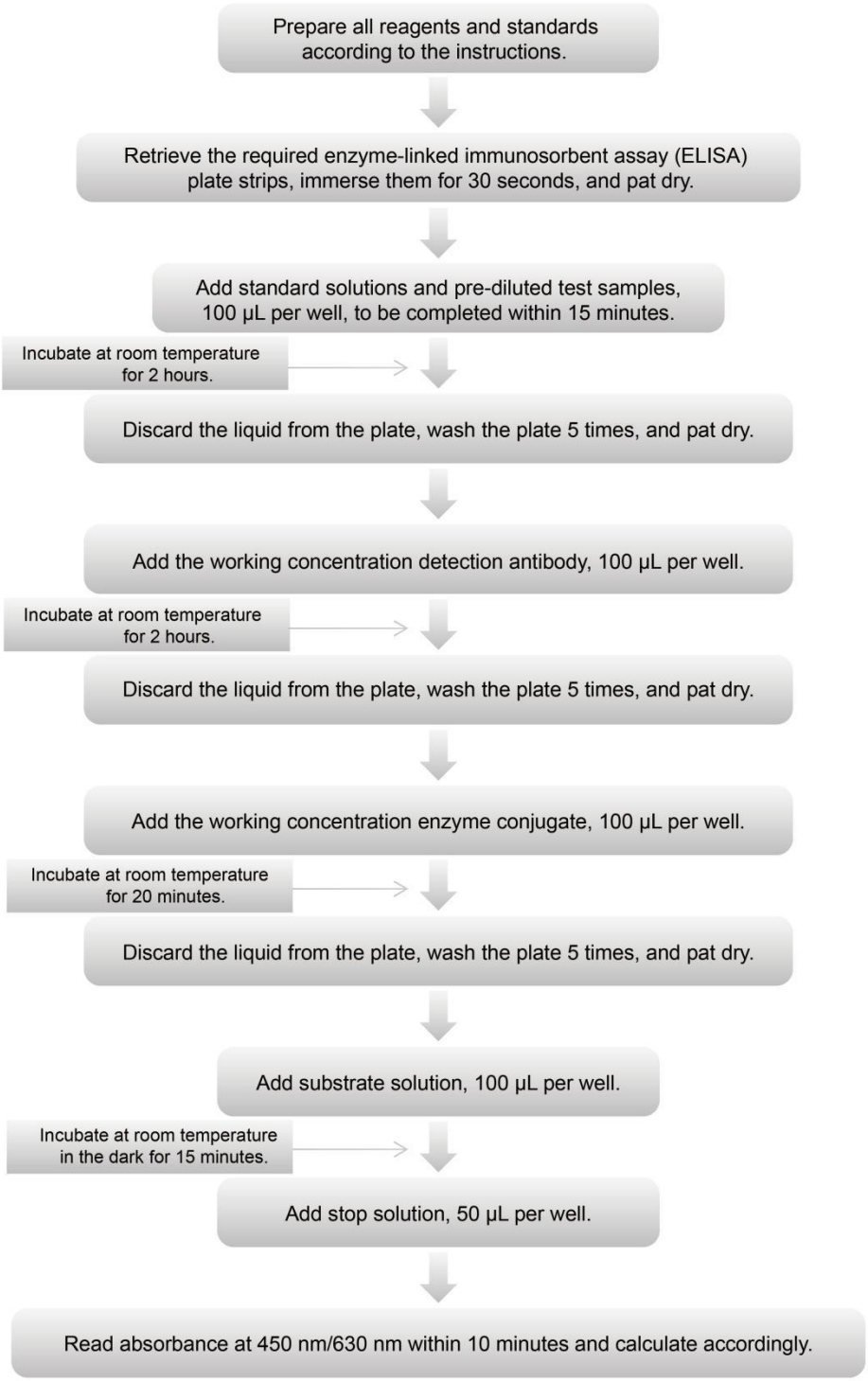
n.d. refers to sample concentrations below the detection range, which is 62.5 pg/mL.

7. Specificity Assay

This assay kit recognizes both natural and recombinant BMP-7, with no observed significant cross-reactivity or interference effects.

Recombinant human:
BMP-2
BMP-3
BMP-4
BMP-5
BMP-6
TGF-β1

Detection Schematic



Frequently Asked Questions

Issues	Causes	Solution
Poor standard curve	Inaccurate pipetting volumes	Check the pipette, calibrate it regularly, handle it carefully, tightly cap the tip while mixing thoroughly, and minimize foam formation.
	Inappropriate dilution methods	
Low color intensity	Insufficient incubation time	Allow sufficient incubation time, and replace samples and reconstituted standards overnight.
	Inadequate pipetting volumes or improper dilution	Calibrate pipettes and standardize operations.
High coefficient of variation (CV)	Improper washing of the ELISA plate	Use the correct washing procedure; if using a plate washer, check all ports for blockages.
	Contaminated washing solution	Prepare fresh washing solution.
Low sensitivity	Improper storage of the assay kit	Store according to the product component table.