Product information

User's Manual

Aminoacyl-tRNA Synthetase Complex-Interacting Multifunctional Protein I (AIMP1) ELISA

For the determination of Aminoacyl tRNA synthetase complex-interacting multifunctional protein 1 in human serum and cell lysate.

REF IB29300

Σ 96

RUO **For research use only, not for use in diagnostic procedures.**

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1. INTENDED USE

This AIMP1 ELISA kit is to be used for the determination of Aminoacyl tRNA synthetase complex-interacting multifunctional protein 1 (AIMP1) in human serum and cell lysate. This kit is intended for research use only, not for use in diagnostic procedures.

2. INTRODUCTION

AIMP1, previously known as p43, is an auxiliary component of ARS multi complex and a precursor of cytokine endothelial-monocyte activating polypeptide II (EMAPII). AIMP1 is known as a cytokine working in the control of angiogenesis, inflammation and wound healing. AIMP1 also has glucagon-like hormonal activity. This AIMP1 ELISA kit is to be used for the determination of AIMP1 in human serum and cell lysate. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for AIMP1. The minimum detectable dose of human AIMP1 using a standard curve generated is 0.29 ng/ml.

3. AIMP1 ELISA KIT COMPOSITION

COMPONENT	DESCRIPTION	STOCK CONCENTRATION	AMOUNT *
Pre-coated 96 Well Strip plate	Anti-AIMP1 mAb	-	
Detection Antibody	Anti-AIMP1 pAb	400 μg/ml	120 μl
Secondary Antibody-HRP	Anti-Rabbit IgG-HRP	100 μg/ml	15 μl
Standard protein	Recombinant human AIMP1	10 μg/ml	20 μl
Sample Diluent	Standard & Sample Dilution	-	10 ml
Assay Diluent	Antibody dilution	-	20 ml
Development reagent	TMB	-	5 ml
Stop Solution	2N H_2SO_4	-	5 ml
Washing Buffer (10 X)	PBS Stock	10X PBS	-

* Amount per 96 wells plate

4. ADDITIONAL MATERIALS REQUIRED

- Distilled or deionized water
- Washing Buffer : 0.05% Tween-20 in 1X PBS (300㎖)
- ELISA Reader : 450nm wavelength

5. STORAGE

Component	Storage
Standard protein	**-20 °C**
Pre-coated 96 well plate	
Detection Antibody	**4 °C**
Secondary Antibody HRP	
Sample Diluent	
Assay Diluent	
Development reagent	
Stop Solution	
Washing buffer 10 X	

Store Standard Protein at -20℃ upon arrival.

6. ASSAY PROCEDURE

All reagents should be equalized with room temperature (18-25 ℃) before use.

1. Preparation of serial diluted standards (40, 20, 10, 5, 2.5, 1.25, 0.625, 0 ng/ml) and samples with sample diluent.

   Note: Dilute the samples with sample diluent, based on the expected concentration of the analyte, to fall within the concentration range of the standards.

   The concentration of standard in stock is 10μg/ml. Prepare 7 tubes containing 400μl standard diluent and produce a serial dilution.

2. Add 100μl of diluted standards and samples (His-AIMP1 Standard & test sample) solution to each well. Incubate at 37℃ for 1 hour. During incubation time to prepare the detector antibody that diluted in Assay diluent (1: 100).

3. Remove the solution in well, and add 200μl of Wash buffer to each well.

   Gently shacking the plate 9~10 times, and remove the Wash buffer. Repeat at least three times. Completely remove the Wash buffer in well.

4. Add 100μl of diluted detection antibody solution to each well. Incubate at 37℃ for 1 hour. During incubation time to prepare Secondary antibody HRP that diluted in Assay buffer (1:1000).

5. Repeat the wash as in step 3.

6. Add 100μl of diluted anti-Rabbit IgG-HRP solution to each well. Incubate for 1 hour at 37℃.

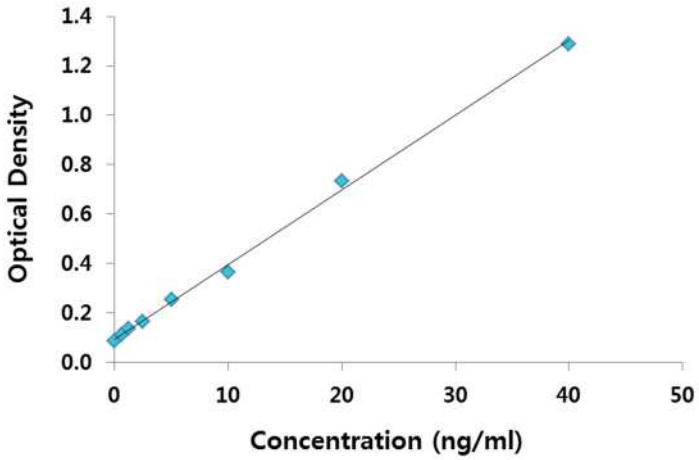
7. Repeat the wash as in step 3.

8. Add 50μl of TMB solution to each well. The liquid in the wells will begin to turn blue. Incubate at room temperature for a proper color development.(10-15 minutes)

9. Add 50μl of Stop Solution to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow. Incubate at room temperature for 2~3 minutes.

10. Read the absorbance of each well at 450nm.

7. STANDARD DATA



8. PRECISION

Intra-assay Precision (Precision within an assay)

The low, medium, high spiked sample was tested four times on one plate to assess intra-assay precision.

AIMP1 Con (ng/ml)	Sample 1	Sample 2	Sample 3	Sample 4	AVR	SD	CV(%) <20%
10	9.07	9.88	9.75	10.37	9.77	0.54	5.5
20	20.37	21.8	21.93	21.68	21.44	0.72	3.36
40	45.34	46.27	49.88	46.15	46.91	2.02	4.31

Inter-assay Precision (Precision between assays)

The low, medium, high spiked sample was tested in four separate assays to assess inter-assay precision

AIMP1 Con (ng/ml)	plate 1	plate 2	plate 3	plate 4	AVR	SD	CV(%) <20%
10	12.2	11	9.8	15	11.99	2.24	18.65
20	24.5	24.7	21.4	28.9	24.89	3.06	12.31
40	39.4	47.4	46.9	54.2	46.98	6.05	12.88

9. RECOVERY

Samples were spiked with 3 different spiked level through the range of the assay and the recovery rates were evaluated by comparing the measured value to the expected amount of AIMP1 in serum samples (n=4).

Spike (ng/ml)	Control	Serum 1	Serum 2	Serum 3	Serum 4	AVR Recovery (n=4)	SD
0	1.0	9.3	6.8	2.9	1.6		
20	14.0	21.2	24.4	20.4	16.4	110.6	19.01
40	31.3	37.7	36.5	41.3	32.5	100.7	15.17
80	62.9	67.5	62.3	61.5	55.5	90.5	3.73

10. Sensitivity

The minimum detectable dose of AIMP1 was determined by two standard deviations to the mean value of 20 blank samples. The minimum detectable dose of human AIMP1 using a standard curve generated is 0.29 ng/ml.

11. Specificity

This kit exhibits no detectable cross-reactivity with GRS, HRS, KRS, NRS, SRS, WRS and YRS.

12. TROUBLESHOOTING

Problem	Solution
Low O.D.	1. Incorrect dilutions or pipetting errors 2. Improper incubation times 3. Improper mixing of the TMB substrate. Each component is mixed in equal parts. 4. Wrong filter on microtiter reader. Wavelength should be 450 nm for TMB, 490 nm for OPD, or 405 nm for ABTS. 5. Kit materials or reagents are contaminated or expired. 6. Incorrect reagents used.
High O.D.	1. Cross contamination from other samples or control 2. Incorrect dilutions or pipetting errors 3. Improper washing 4. Wrong filter on microtiter reader. Wavelength should be 450 nm for TMB, 490 nm for OPD, or 405 nm for ABTS. 5. Contaminated buffers or enzyme substrate 6. Improper incubation times 7. Kit materials or reagents are contaminated or expired.
Poor Duplicates	1. Poor mixing of specimens 2. Incorrect dilutions or pipetting errors 3. Inconsistency in following ELISA protocol 4. Inefficient washing
High Background	1. Contaminated buffers or enzyme substrate 2. Incorrect dilutions or pipetting errors 3. Kit materials or reagents are contaminated or expired. 4. Inefficient washing
None Development	1. Procedure not followed correctly 2. Contaminated buffers, enzyme substrate or conjugate 3. Kit materials or reagents are contaminated or expired.

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13. REFERENCES

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