KAMIYA BIOMEDICAL COMPANY

Chicken MRP-126 ELISA

For the quantitative determination of MRP-126 in chicken plasma and feces

Cat. No. KT-1910

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT

The **K-ASSAY®** Chicken MRP-126 ELISA is an enzyme immunoassay for the quantitative determination of MRP-126 in chicken plasma and feces. For research use only.

INTRODUCTION

MRP-126 belongs to the calgranulin protein family. It is a homodimer that calcium dependently sequesters zinc, thereby inhibiting bacterial growth. Proteomic studies indicate that its levels increase in chicken cecum following salmonella infection. In studies, we have found that fecal MRP-126 levels increase up to 40-fold after bacterial infection.

PRINCIPLE

Diluted samples (fecal extracts or plasma) and calibrators are incubated in microtiter wells for 45-minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. MRP-126 molecules are thus sandwiched between immobilized antibody and the HRP conjugate. The wells are then washed to remove unbound HRP-labeled antibodies. TMB is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of MRP-126 is proportional to the absorbance and is derived from a calibration curve.

COMPONENTS

- Anti-MRP-126 coated 96-well plate (12 x 8-well strips)
- Anti-MRP HRP Conjugate,11 mL
- MRP-126 Stock. **Store at -20℃.**
- 20X Wash Solution, 50 mL
- Diluent, 50 mL
- TMB Reagent, 11 mL
- Stop Solution (1N HCl), 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes and tips
- Distilled or de-ionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader with an optical density range of 0-4 at 450 nm
- Graphing software

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (25℃) before use.
3. Optimum results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20X stock. Prior to use, dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water.

DILUENT PREPARATION

The diluent is supplied ready to use. Do not substitute other buffers. It has been specially formulated for measurement of MRP-126.

CALIBRATOR

1. Thaw the MRP-126 just before use. After use, freeze it at or below -20℃ if future use is intended.
2. Label eight 1.5 mL microcentrifuge tubes as 10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0 ng/mL.
3. Into the tube labeled 10 ng/mL, pipette the volume of diluent detailed on the MRP-126 stock vial label. Then add the indicated volume of MRP-126 stock and mix gently. This provides the 10 ng/mL calibrator.
4. Dispense 250 µL of diluent into the tubes labeled 5, 2.5, 1.25, 0.625, 0.313, 0.156, and 0 ng/mL.
5. Prepare the 5 ng/mL calibrator by diluting and mixing 250 µL of the 10 ng/mL calibrator with 250 µL of diluent in the tube labeled 5 ng/mL.
6. Similarly prepare the 2.5 to 0.156 ng/mL calibrators by two-fold serial dilution.

SAMPLE PREPARATION

Plasma

We found MRP-126 levels ranging from 0.1 to 1 µg/mL. Optimal dilutions should be determined empirically, but we suggest testing at an initial dilution of 100-fold. That can be obtained by mixing 5 µL of plasma with 495 µL of diluent. To avoid matrix effects do not test dilutions less than 20-fold.

Feces

We recommend that feces be extracted and prepared as follows.

1. Accurately weigh approximately 100 mg of feces into a tared 1.5 mL microcentrifuge tube.
2. Add 9 volumes of 10 mM Tris, 150 mM NaCl pH 7.5 (i.e., 0.9 mL to 100 mg of feces).
3. Vortex several times over a 30-minute period to prepare a suspension that is as homogeneous as possible.
4. Centrifuge in a microcentrifuge (5 minutes at 15,000 rpm).
5. Save the supernatant. This represents a 10-fold “dilution” of the fecal sample. Samples may be stored frozen at or below -20℃.

We found MRP-126 levels in feces ranging from 0.1 to 12.5 µg/g. Optimal dilutions should be determined empirically, but we suggest testing at an initial dilution of 200-fold. That can be obtained by mixing 15 µL of the 10-fold diluted sample (step 5, above) with 285 µL of diluent.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the cassette. Store unused strips at 4℃ in a sealed bag with desiccant.
2. Dispense 100 µL of calibrators and diluted samples into appropriate wells.
3. Incubate on a plate shaker at 150 rpm / 25℃ for 45-minutes.
4. Aspirate the contents of the microtiter wells and wash the wells five times with 1x wash solution using a plate washer (400 µL/well).
5. If necessary, strike the wells sharply onto absorbent paper to remove residual wash solution.
6. Add 100 µL of HRP conjugate into each well.
7. Incubate on a plate shaker at 150 rpm / 25℃ for 45-minutes.
8. Wash as detailed above.
9. Dispense 100 µL of TMB into each well.
10. Incubate on a plate shaker at 150 rpm / 25℃ for 20-minutes.
11. Stop the reaction by adding 100 µL of Stop Solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Measure absorbance at 450 nm with a microtiter plate reader within five minutes.

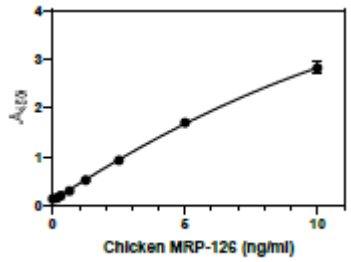
CALCULATION OF RESULTS

1. Using curve fitting software, construct a calibration curve by plotting absorbance values of the calibrators versus concentration.
2. Fit the calibration curve to and appropriate model and determine the concentration of the samples from the calibration curve. We use a second order polynomial (quadratic) fit.
3. Multiply the derived concentration by the dilution factor to determine the concentration in the serum sample.
4. If the A_{450} values of samples fall outside or at the extremes of the calibration curve, samples should be diluted appropriately and re-tested.

TYPICAL CALIBRATION CURVE

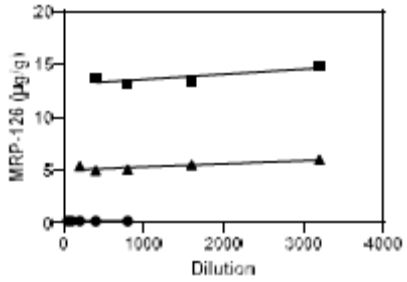
A typical calibration curve is shown below. This curve is for illustration only and should not be used to calculate unknowns.

MRP-126 (ng/mL)	A450
10	2.823
5	1.697
2.5	0.922
1.25	0.514
0.625	0.292
0.313	0.196
0.156	0.148
0	0.128



ASSAY PERFORMANCE

Parallelism: To assess performance of the assay, three extracts of feces that had MRP-126 content ranging from 0.17 to 14.4 µg/g, were serially diluted to produce values within the dynamic range of the assay.



STORAGE

The MRP-126 stock should be stored in a freezer at or below -20℃ when the kit is received. The rest of the kit should be stored in a refrigerator at 4℃ and should not be frozen. The kit will remain stable until the expiration date provided that the components are stored as described. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

FOR RESEARCH USE ONLY

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