Chicken MRP-126 ELISA
Life Diagnostics, Inc., Catalog Number: MRP-5

INTRODUCTION

MRP-126 belongs to the calgranulin protein family. It is a homodimer that calcium dependently sequesters zinc, thereby inhibiting bacterial growth (ref 1). Proteomic studies indicate that its levels increase in chicken cecum following salmonella infection (ref 2). In studies at Life Diagnostics, we have found that fecal MRP-126 levels increase up to 40-fold after bacterial infection.

PRINCIPLE OF THE ASSAY

Diluted samples (fecal extracts or plasma) and standards 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 standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-MRP-126 coated 96-well plate (12 x 8 well strips)
- Anti-MRP HRP Conjugate, 11 ml
- MRP-126 Stock. **Store at -20°C**
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: MRPD50-1, 50 ml
- TMB Reagent: TMB11-1, 11 ml
- Stop Solution (1N HCl): SS11-1, 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized 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

STORAGE

The MRP-126 stock should be stored in a freezer at or below -20°C when the kit is received. The rest of the kit should be stored in a refrigerator at 4°C and should not be frozen. The kit will remain stable for at least six months from the date of purchase 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.

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°C) before use.
3. Optimum results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

DILUENT PREPARATION

The MRPD50-1 diluent is supplied ready to use. Do not substitute other buffers. MRPD50-1 has been specially formulated for measurement of MRP-126.

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 deionized water.

STANDARD PREPARATION

1. Thaw the MRP-126 just before use. After use, freeze it at or below -20°C 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 MRPD50-1 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 standard.
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 standard by diluting and mixing 250 µl of the 10 ng/ml standard with 250 µl of diluent in the tube labeled 5 ng/ml.
6. Similarly prepare the 2.5 to 0.156 ng/ml standards 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 MRPD50-1 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°C.

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 MRPD50-1 diluent.

Life Diagnostics, Inc., P.O. Box 5205, West Chester, PA 19380
610-431-7707 – 610-431-7818 (Fax)
info@lifediagnostics.com – www.lifediagnostics.com

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the cassette. Store unused strips at 4°C in a sealed bag with desiccant.
2. Dispense 100 µl of standards and diluted samples into appropriate wells.
3. Incubate on a plate shaker at 150 rpm / 25°C 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°C 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°C 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.

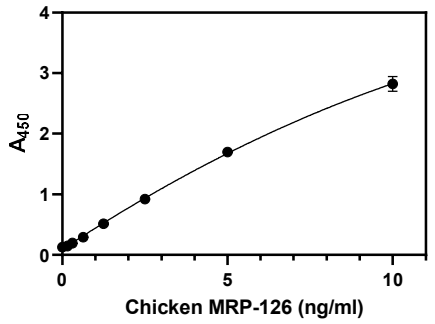
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.
2. Fit the standard curve to and appropriate model and determine the concentration of the samples from the standard 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 standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

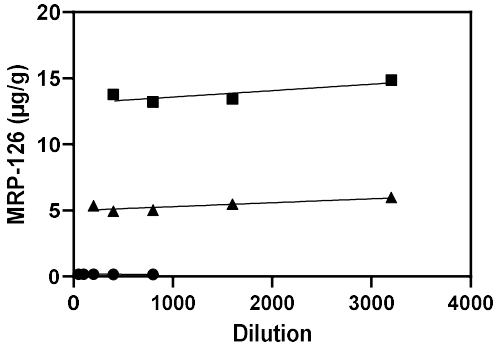
A typical standard curve is shown below. This curve is for illustration only and should not be used to calculate unknowns.

MRP-126 (ng/ml)	A_{450}
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.



REFERENCES

1. Bozzi AT and Nolan EM. Avian MRP126 restricts microbial growth through Ca(II)-dependent Zn(II) sequestration. Biochemistry. 59(6): 802-817 (2020)
2. Matulova M., et.al. Chicken innate immune response to oral infection with Salmonella enterica serovar Enteritidis. http://www.veterinaryresearch.org/content/44/1/37 (2013)

Rev 12162021

For technical assistance please email us at
techsupport@lifediagnostics.com