Cat. No. AEFF1411-25

For Research Use Only

FD AZMA Chicken, Cattle and Pig Kit

Azma Elixir Pajooh

PCR kit for the qualitative detection of Chicken, Cattle and Pig.

For 25 Reactions

Store at -20 °C.

INTRODUCTION

In many countries worldwide, there are legislations for use, meat of chicken, cattle and pig in processed food. Hence, reliable methods for the detection and identification of species of meat in food and feed are required.

With the **FD AZMA Chicken, Cattle and Pig Kit** series, AZMA ELIXIR offers a wide range of easy and reliable assays for the detection of meat of these species in food. The **FD AZMA Chicken, Cattle and Pig Kit** allow a fast, safe and easy detection in food and feed samples.

USAGE

The **FD AZMA Chicken, Cattle and Pig Kit** is designed to detect the mitochondrion specific gene of chicken, cattle and pig in various processed food, raw material, feed and etc.
This kit provides PCR MasterMix and the specific primer set for rapid testing by Conventional PCR, as well as the positive Control for reliable results.

CONTENTS

This kit is intended for **25** reactions, including positive controls, **table 1**.

Table 1. Kit Contents

Reagent	Volume	Description
PCR Azma Master mix	750 µl	2X
Chicken F primer	25 µl	Ready to use
Chicken R primer	25 µl	Ready to use
Cattle F primer	25 µl	Ready to use
Cattle R primer	25 µl	Ready to use
Pig F primer	25 µl	Ready to use
Pig R primer	25 µl	Ready to use
Chicken Positive control	15 µl	DNA
Cattle Positive control	15 µl	DNA
Pig Positive control	15 µl	DNA
PCR grade DDW	1 ml	PCR Grade water

PRECAUTIONS

· Store extracted positive material (samples and positive controls) away from all other reagents and add it to the reaction mix in a separate area.
· Thaw all components thoroughly on ice before starting experiment.
· When thawed, mix the components and centrifuge briefly.
· Do not use a kit after its expiration date.

· Use disposable gloves, laboratory coats and eye protection while handling samples and reagents. Thoroughly wash hands afterwards.
· Specimens should be considered potentially infectious and handled in biological cabinet in accordance with appropriate biosafety practices.
· Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
· Avoid contact of specimens and reagents with the skin, eyes and mucosa. If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
· Use of this product should be limited to personnel trained in the techniques of DNA amplification.

METHODS AND PROCEDURES

A. Preparing the PCR

To prevent the risk of contamination with foreign DNA, we recommend that all experiment steps be Performed in a PCR clean room or separated environment area. Filter tips are recommended for each step.
Thawing the kit components on ice. Using ice is recommended during experiment for maintaining the enzyme activity.
Total reaction volume is 20 µl the volume of DNA sample is 2 µl. Prepare a reaction mixture with specific mix primer for each target gene according to table 2.

Table 2: PCR reaction mixture

Composition	Volume
PCR Azma Master mix	10 µl
Mix primer	2 µl
DDW	6 µl
Template	2 µl
Total Volume	20 µl

CONTROL + Positive control amplification: Add 0.5 to 1 µl of Control DNA instead of sample DNA.

CONTROL - Negative control amplification: Add 2 µl of H2O PCR-grade instead of sample DNA.

Mix the reagents in the PCR reaction tubes by tapping minimum of 5 times. Briefly centrifuge the tubes

B. Amplification

- Program your PCR instrument according to manufacturer's manual.

Create a temperature time profile on your instrument as follows in Table 3.

Table 3: Temperature Time Profile

Temperature	Time	Cycle
95	5 min	1
95	30 s	40
60	30 s	
72	30 s	
72	5 min	1

C. Agarose Gel

1. Use 1.5 % standard agarose gel.
2. Directly load 5 µl for each PCR into a separate lane. The loading buffer is included in the PCR mixture.
3. Stop electrophoresis after migration of 2.5 cm (depending on the electrophoresis chamber used, e.g., run for 25 minutes at 100 V).

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D. Results

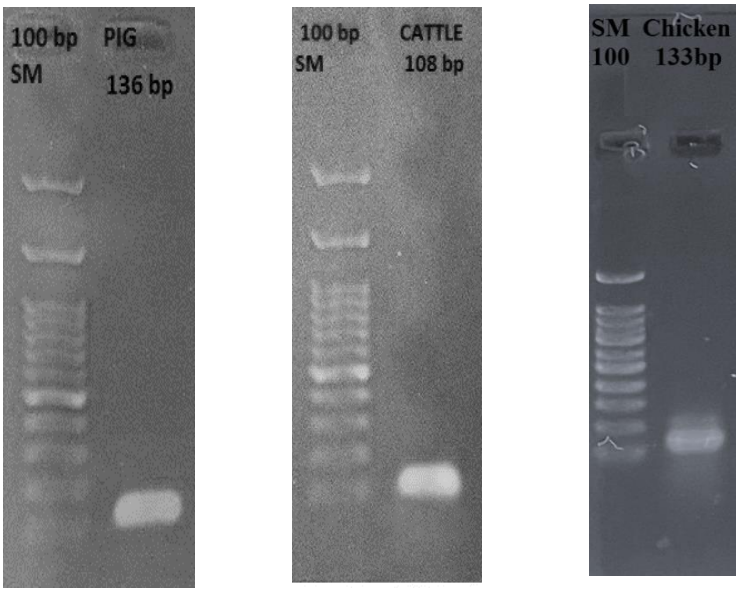
Gel Evaluation

- The positive controls show band according to the table 4.

Table 4: Molecular Weight of target genes.

Target Gene	MW (bp)
Chicken	133
Cattle	108
Pig	136

Figure 1. Sample gel image of a reaction using the recommended cycling conditions



E. Troubleshooting

1. No signal in positive control lane: Annealing temperature is too high. Use recommended annealing temperature and make sure that your cycler is calibrated and the temperature on the display is the actual block temperature.

2. Too many bands: Annealing temperature is too low, increase annealing temperature gradually. This could also be due to PCR mis-priming prior to cycling. Make sure your PCR reaction tubes are kept cool to avoid priming before cycling. The initial cycles are critical. Alternatively, use a hotstart Taq Polymerase.

3. Negative control shows a PCR product: This is due to contamination of either the master mix, the water template used in the negative control tube, or the pipette tip used to mix the negative control reaction mixture.