# microzone®

# Avian GEN ID kit

P. Code	Size in 100 Rxn	Component	Description	Lot Number	Expiry
2MLP-50	2 x 1 mL	microLYSIS Plus	Direct to PCR lysis buffer for tough cells.		
2MMAGID-1	1 x 1 mL	Avian GEN ID Mastermix	2X concentrated, hot-start Taq, red loading dye, inhibi- tor-resistant mastermix, containing P0/P2/P8 primers.		
AFPC-0.05	1 x 50 μL	Avian Female Positive Control	DNA from female Gallus gallus domesticus		
AMPC-0.05	1 x 50 μL	Avian Male Positive Control	DNA from male Gallus gallus domesticus		
5JWA-1	1 x 1 mL	Just water	Molecular Grade Water		

### Applications

• Rapid determination of Avian Sex.

## **Product Description**

Microzone's Avian GEN ID kit allows rapid avian sexing results from feather or blood samples. It utilizes the P0/P2/P8 primer set developed by Han et al. in 2009, which targets specific regions of the avian sex chromosomes (CHD1 gene).

The Avian GEN ID kit includes microLYSIS Plus as a direct-to-PCR lysis buffer to efficiently release DNA from bird feathers or blood. The inhibitor-resistant Avian GEN ID Mastermix contains P0/P2/P8 primers and is optimised for the swift amplification of the CHD1 gene from lysates prepared in microLYSIS Plus. After endpoint PCR, samples can be directly loaded onto a gel due to the red agarose loading dye incorporated in the Avian GEN ID Mastermix.

# Protocol

### Lysis

Feather—Cut 1 to 3 mm of 1 to 3 calamus and place into 20  $\mu$ L of microLYSIS Plus in a PCR tube.

**Blood**—Cut 1 to 2 mm<sup>2</sup> of dried blood spot and add to 20  $\mu$ L of microLYSIS Plus in a PCR tube.

Transfer the PCR tube to a thermal cycler and run the following profile:

Temperature	Time
65°C	15 minutes
96°C	2 minutes
65°C	4 minutes
96°C	1 minute
65°C	1 minute
96°C	30 seconds

### **PCR Setup**

Set up PCR reaction as described below:

Components	Volume
2X Avian GEN ID Mastermix	10 µL
Lysate	1 to 2 μL
Just Water (Molecular Grade Water)	x μL (up to 20 μL)

Key Features

- Simple—direct-to-PCR makes extraction and setup easy.
- Versatile—get reliable results for many species from blood and feather.
- Rapid—from collection to result in less than 3 hours.



*Gallus gallus domesticus* samples were used as template for PCR amplification with Avian GEN ID Mastermix. Avian sex was determined by the presence of two fragments (female) or one fragment (male).

In the place of the lysate, include:

For Negative Control: 2 µL Just Water

For Female Positive Control: 2 µL Avian Female Positive Control

For Male Positive Control: 2  $\mu$ L Avian Male Positive Control

# Thermocycling

Transfer the reactions to the thermal cycler and set as follows:

Cycles	Temperature	Time
1	95°C	5 min
	95°C	15 sec
35	55°C	15 sec
	72°C	30 sec
1	72°C	5 min

# **Gel Electrophoresis**

Load 10  $\mu\text{L}$  samples onto a 1.5% agarose gel with appropriate ladder and run for 45 minutes at 120 Volts.

# Results

Male - a single ~350 bp fragment.

Female - either two or three fragments: one ~350 bp, one ~380 bp (in some species these are too similar to differentiate) and one ~480 bp.

For research use only

### **Product Handling**

### Storage

To ensure the quality of the product until the expiry date keep at the recommended storage temperature and limit exposure to light.

### **Contamination Control**

To prevent erroneous results ensure work environment is free of contamination by cleaning your workstation and equipment with a DNA decontaminant daily, wear gloves, use sterile tubes and filter pipette tips.

Simple | Effective | Efficient