



Ready To Use PCR Reagents

AVIAN DNA GENDERING

Cat. No. 60ADS100

INSTRUCTION MANUAL

I. Intended Use

ADG Ready to Use PCR Reagents are intended for determination of avian gender by PCR amplifications. All reagents are ready to use for a successful amplification, from DNA extraction to obtaining PCR products suitable for loading onto Agarose gel.

II. General Information

Each package contains **Rapid One Step Blood Extraction Buffer** (Tube **A**), which is intended for use with fresh or dry blood samples. The extraction step yields appropriate amount of crude DNA needed for a successful amplification of **ADG** via PCR. No purification is needed!

Tubes **B**, **C** and **D** are the components for subsequent use in PCR amplification. Tube **B** contains **ADG-PCR mix**, Tube **C** contains **ADG Activation Buffer** and Tube **D** contains the **Positive Control**. The Extraction Buffer (Tube **A**) also serves as **Negative Control**. Also included are **Tissue/Swab Extraction Buffer** (Tube **E**) and **Tissue/Swab Neutralization Buffer** (Tube **F**). Each PCR set up should include 3 reaction vials; each vial should be added with: **5µl ADG-PCR mix**, **10µl ADG Activation Buffer** and **5µl DNA product of the Extraction step / Positive Control/ Negative Control**. Following the addition and mixing of all the above ingredients, the reaction vials are placed in thermal cycler for amplification according to the program detailed in the Step by Step chapter (see section VIII). At the end of the program the product should be visualized on a 3-4% Agarose gel, yielding a **250-500bp** band.

III. Description Of Avian Sexing

The majority of parrot species, as many of the avian species are considered mono-morphic, as their sexual identity is undistinguishable phenotypically. Sex in birds is assigned genetically: the female is the heterogametic sex (ZW) and the male is the homogametic sex (ZZ). Therefore, it can be assumed that some genetic sequences occur in females but not in males. The gene-encoding chromo-helicase-DNA binding protein 1 (CHD-1) has been found on the Z and W chromosomes; however, the alleles are not identical, allowing differentiation between males and females, since the CHD-1 gene has at least one intron of different sizes on the Z and W alleles. Multiple primers have been designed to take advantage of that character of the CHD-1 gene. PCR technique application enables easy and rapid detection of gender in most avian species.

IV. Sex Determination

Using a mixture of primers from the CHD-1 gene it is possible to determine the gender of most avian species. To determine the sex of the bird it is necessary to extract genomic DNA from the bird. The most common source for gDNA comes from blood samples (dried or fresh), freshly plucked feathers or blood from egg shell vessels. The determination is done on a 3-4% Agarose gel yielding **one band for male** and two bands at a **10-50 bp distance for female** ranging at about **250bp to 500bp**.

V. Contents (Sufficient for 48 tests)

Tube A	Rapid One Step Blood Extraction Buffer
Tube B	ADG-PCR mix (Green cap)
Tube C	Specific ADG Activation Buffer (Blue cap)
Tube D	Specific ADG Positive Control (Red cap)
Tube E	Tissue/Swab Extraction Buffer
Tube F	Tissue/Swab Neutralization Buffer
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VI. Essentials Not Included

RNAase free PCR reaction vials.
 PCR Thermo-Cycler.
 5-10µ, 100µl Pipettes and filter tips.
 Micro-centrifuge.
 Vortex.
 Heating bath or heating block.
 Agarose, DNA size marker.
 Microwave for Agarose casting.
 Horizontal Mini-Electrophoresis chamber, Comb and power pack.
 TBE /TAE Buffer and Ethidium Bromide (EB).
 UV Transilluminator (254nm for EB).
 A pair of sterile scissors.
 A cutter (for swab application).

VII. Storage And Handling

- Store at 4°C for 6 months or at -20°C for two years.
- Use gloves and maintain clean working conditions.
- Avoid spillage and cross contamination of solutions
- Change tips between reagents and between reaction vials.
- Disinfect scissors before and after each cutting of blood filters.
- Do not mix reagents from different batches.
- Always treat samples with precaution, and dispose as biological material.
- Remember that Ethidium Bromide is hazardous, and use the UV transilluminator carefully.
- It is recommended to incinerate the contents after use.

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VIII. Step By Step Protocol

Blood Extraction:

- (1) Into an empty clean vial, add **100µl of Rapid OneStep Blood Extraction Buffer (Tube A)** for **every 5µl** of fresh blood sample or approximately 3/5 mm² piece of Whatman/tissue paper soaked with dry blood. Make sure the piece of paper is submerged underneath the extraction buffer.
- (2) Incubate samples at **50°C** for **10 minutes** followed by a subsequent **95°C** for additional **10 minutes**.
- (3) Centrifuge sample at **>10,000 rpm** for **1 minute** to allow the paper and cell debris to pellet. The extracted DNA product is in the liquid phase, ready to be used for PCR.

Feather Extraction:

- (1) Cut de lower portion (root) of freshly plucked feather (5mm) and insert it into a clean 1.5 ml vial.
- (2) Add **100µl of Tissue/Swab Extraction Buffer (Tube E)**. Make sure the sample is submerged underneath.
- (3) Incubate sample at **95°** for **10 minutes**.
- (4) Centrifuge sample at **>10,000 rpm** for **1 minute** and transfer supernatant to a new clean 1.5 ml vial.
- (5) Add **100µl of Tissue/Swab Neutralization Buffer (Tube F)**, briefly vortex mixture and the product will be ready for PCR use.

Extracted DNA product (of any source)* may be applied immediately for PCR or stored for a few days at 4°C / several months at -20°C. Please mark the vial properly for future identification.

*Note: **The reagents have been adjusted for use with crude DNA extraction to enable better sensitivity (with less handling).**

PCR Procedure:

- (1) Into a clean reaction vial add: **5µl ADG-PCR mix (Tube B)**, **5µl** of the **Extracted DNA product** and **10µl** of the specific **ADG-Activation Buffer (Tube C)**. Mark each reaction vial properly to avoid mistakes.
- (2) Into a second clean reaction vial add **5µl ADG-PCR mix (Tube B)**, **5µl** of the **Positive Control (Tube D)** and **10µl** of the specific **ADG Activation Buffer (Tube C)**. Mark this vial as Positive Control reaction.
- (3) Into a third clean reaction vial add **5µl ADG-PCR mix (Tube B)**, **5µl** of the **Extraction Buffer (Tube A)** and **10µl** of the specific **ADG Activation Buffer (Tube C)**. Mark this vial as **Negative Control** reaction.
- (4) Gently mix each reaction vial (do not vortex!) and place in the thermal cycler for amplification.

ADG Specific PCR Program:

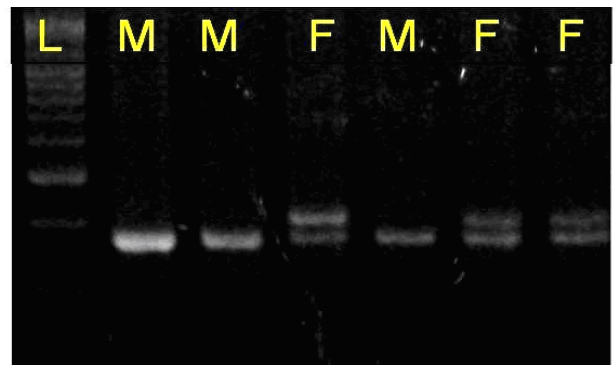
- 95°C for 3 minutes**
- 20 cycles of**
 - 94°C for 40 seconds**
 - 52°C for 30 seconds**
 - 72°C for 20 seconds**
- 18 cycles of**
 - 94°C for 20 seconds**
 - 50°C for 10 seconds**
 - 72°C for 20 seconds**
- End cycles**
 - 72°C for 2 minutes**
 - Stop at 8°C**

- (5) If not used immediately, store PCR products at 4°C until application on Agarose.

IX. Reading And Interpreting The Results

- Visualize PCR products on **3-4% Agarose gel**, along with a size marker (see Fig. 1).
- Mark each well and load the whole content of each reaction vial into the relevant wells.
- Electrophoresis should be very slow to enable visualization of 10-50bp differences between samples.
- The Positive Control driven from a female African gray parrot should yield 2 **250-500bp** sized bands (with **10-50bp** intervals)
- No band should be detected at the Negative Control lane.
- A male expected product should be a single band of **250-500bp** size.

Fig. 1 - Visualization of the PCR product.



Lanes: L - Size Marker, M - Male, F - Female/Positive Control.

X. Limitations And Troubleshooting

- For *in vitro* use only. Do not use internally or externally in humans or animals.
- A false result may occur, even if precaution has been taken. To eliminate inconclusive results, always use the Negative Control in each PCR set.

XI. References

- Chang HW et al. (2008) High-throughput avian molecular sexing by SYBR green-based real-time PCR combined with melting curve analysis. BMC Biotechnol. 12;8:12.
- Cortés O et al. (1999) Avian sexing: an optimized protocol using polymerase chain reaction-single-strand conformation polymorphism. J Vet Diagn Invest. 11(3):297-9.
- Griffiths R et al. (1997) Sex identification in birds using two CHD genes. M. Proc Biol Sci. 22; 263(1374):1251-6. Erratum in: Proc R Soc Lond B Biol Sci 1997 22; 264(1381):623.
- Sacchi P et al. (2004) A non-invasive test for sex identification in short-toed Eagle (Circaetus gallicus). Mol Cell Probes 18(3):193-6.
- Soderstrom K et al. (2007) A minimally invasive procedure for sexing young zebra finches. J Neurosci Methods. 15;164(1):116-9. Epub 2007 Apr 18.

For further information and assistance please contact your local distributor or Biogal Galed Labs. Directly by e-mail: info@biogal.co.il or by tel: 972-4-9898605 / fax: 972-4-9898690.