Ready To Use PCR Reagents

AVIAN SPECIFIC DNA CONTROL

Cat. No. 60ACO100
INSTRUCTION MANUAL

I. Intended Use

ACO Ready to Use PCR Reagents are intended for amplifications of avian DNA. All reagents are ready to used 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 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 **DNA** via PCR. No purification is needed! Tubes **B**, **C** and **D** are the components for subsequent use in PCR amplification. Tube **B** contains **PCR mix**, Tube **C** contains **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 PCR mix**, **10µl 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 VI). At the end of the program the product should be visualized on 1.5% Agarose gel, yielding a **400bp** band. If a band is visualized at the correct size, the DNA extracted is at a sufficient quality for PCR amplification using Biogal *Ready to Use PCR Reagents.*

III. Contents (Sufficient for 48 tests)

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

RNAase free PCR reaction vials.
PCR Thermo-Cycler.
5-10µ, 100µl Pipettes and filter tips.
Micro-centrifuge.
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 Ethydium Bromide (EB).
UV Transilluminator (254nm for EB).
A pair of sterile scissors.
A cutter (for swab application).

V. 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.

Developed by Karnieli Ltd.

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VI. 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 mm^2 piece of Whatman/ tissue paper soaked with 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.

Tissue/Swab Extraction:

(1) Into a clean 1.5 ml vial add **300µl** of **Tissue/Swab Extraction Buffer** (Tube **E**).

(2) Carefully cut the agar-free swab close to its cotton edge and insert it into the vial. The swab should fit entirely inside the vial, must be covered with buffer and the cap should close easily.

(3) Incubate the swab within buffer **E** for **10 minutes at 95°c.**

(4) Add **300µl** of **Tissue/Swab Neutralization Buffer** (Tube **F**) 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 PCR mix** (Tube **B**), **5µl** of the **Extracted DNA product** and **10µl** of the specific **Activation Buffer** (Tube **C**). Mark each reaction vial properly to avoid mistakes.

(2) Into a second clean reaction vial add **5µl PCR mix** (Tube **B**), **5µl** of the **Positive Control** (Tube **D**) and **10µl** of the specific **Activation Buffer** (Tube **C**). Mark this vial as Positive Control reaction.

(3) Into a third clean reaction vial add **5µl PCR mix** (Tube **B**), **5µl** of the **Extraction Buffer** (Tube **A**) and **10µl** of the specific **Activation Buffer** (Tube **C**). Mark this vial as **Negative Control** reaction.

(4) Gently mix each reaction vial and place in the thermal cycler for amplification.

PCR Program:

A. 95°C for 2 minutes

38 cycles of:

B. 94°C for 30 seconds
C. 55°C for 30 seconds
D. 72°C for 30 seconds

End cycles

E. 72°C for 2 minutes
F. Stop at 8°C

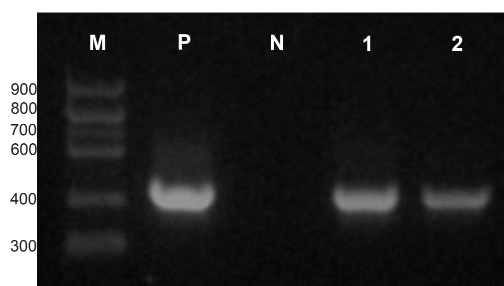
(5) If not used immediately, store PCR products at 4°c until application on Agarose.

VII. Reading And Interpreting The Results

Absence of a band at any height indicate the DNA sample is not at adequate quality for testing and interpreting. Degraded DNA might appear as a smear and indicate the DNA sample is in poor quality and not valid for use. If a high molecular weight band appears, please dilute sample at 1:5 with **Buffer A** and recheck.

- Visualize PCR products on a 1.5% Agarose gel, along with a size marker (see Fig. 1).
- Mark each well and load the whole content of each reaction vial to the relevant wells.
- To each reaction vial, add 6µl Loading Buffer, before lauding on the Agarose gel.
- A sample with adequate level of quality will yield a band of **400bp**.

Fig. 1 - Visualization of the PCR product.



VIII. Limitations And Troubleshooting

- For *in vitro* use only. Do not use internally or externally in humans or animals.

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.