



# Ready To Use PCR Reagents

## AVIAN SALMONELLA spp.

Cat. No. 60ASS100  
INSTRUCTION MANUAL

### I. Intended Use

**ASS Ready to Use PCR Reagents** are intended for avian Salmonella 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 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 ASS via PCR. No purification is needed! Tubes B, C and D are the components for subsequent use in PCR amplification. Tube B contains **ASS-PCR mix**, Tube C contains **ASS 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 ASS-PCR mix**, **10µl ASS 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 1.5% Agarose gel, yielding a **370bp** band.

### III. Description Of The Disease

Salmonella species are gram negative, aerobic, rod-shaped, zoonotic bacteria that can infect people, birds, reptiles, and other animals. This genus includes approximately 2000 species divided into five subgenera. Subgenus I is the most common subgenera found in birds. Subgenus III, containing the species Salmonella arizonae and arizona hinshawii, has occasionally been reported in birds (particularly those that are in contact with reptiles). Transmission of this organism from one host to another is primarily through the air. The bacteria are shed from an infected bird in nasal and or ocular secretions, fecal material, and feather dust. General symptoms of Salmonella include lethargy, anorexia and diarrhea. In chronic cases, arthritis (particularly in pigeons) may be present. Heavily infected birds show excessive thirst, conjunctivitis along with evidence of liver, spleen, kidney or heart damage involvement.

### IV. Diagnosis Of The Disease

The common method of diagnosis is culturing and identifying the bacteria. This method has a few drawbacks such as culturing period while degree of identity provided is low. PCR can rapidly and sensitively identify Salmonella infection and sequencing or specific priming can identify between species. Swab samples should be taken from the cloacae and nasal discharge. In many cases, the bacteria can be identified in whole blood samples as well.

### V. Contents (Sufficient for 48 tests)

Tube A	<b>Rapid One Step Blood Extraction Buffer</b>
Tube B	<b>ASS-PCR mix (Green cap)</b>
Tube C	<b>Specific ASS Activation Buffer (Blue cap)</b>
Tube D	<b>Specific ASS Positive Control (Red cap)</b>
Tube E	<b>Tissue/Swab Extraction Buffer</b>
Tube F	<b>Tissue/Swab Neutralization Buffer</b>
	<b>ASS Instruction Manual</b>

### VI. 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 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

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### 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<sup>2</sup> 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 ASS-PCR mix (Tube B)**, **5µl of the Extracted DNA product** and **10µl of the specific ASS-Activation Buffer (Tube C)**. Mark each reaction vial properly to avoid mistakes.
- (2) Into a second clean reaction vial add **5µl ASS-PCR mix (Tube B)**, **5µl of the Positive Control (Tube D)** and **10µl of the specific ASS Activation Buffer (Tube C)**. Mark this vial as Positive Control reaction.
- (3) Into a third clean reaction vial add **5µl ASS-PCR mix (Tube B)**, **5µl of the Extraction Buffer (Tube A)** and **10µl of the specific ASS 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.

### PCR Program:

**A. 95°C for 2 minutes**

**38 cycles of:**

**B. 94°C for 30 seconds**

**C. 56°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.

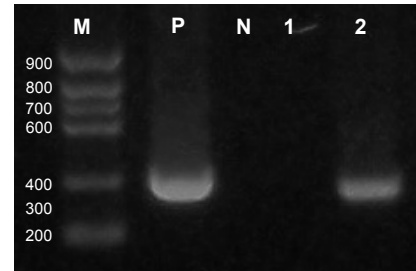
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## IX. Reading And Interpreting The Results

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- Visualize PCR products on 1.5% 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.
- The Positive Control should yield a single band at **370bp**.
- No band should be detected at the Negative Control lane.
- The expected product should be a single band at **370bp**.

**Fig. 1 - Visualization of the PCR product.**



Lanes: M- Size Marker, P- Positive Control, N- Negative Control  
Lanes 1 - 2 are test samples of which 2 is positive for ASS.

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## X. Limitations And Troubleshooting

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- For *in vitro* use only. Do not use internally or externally in humans or animals.
- A false positive result may occur, even if precaution has been taken. To eliminate inconclusive results, always use the Negative Control in each PCR set.
- To avoid false positive interpretation, check vaccination schedules. PCR may be positive 2-6 weeks post vaccination.

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## XI. References

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- Myint MS et al. (2006) The effect of pre-enrichment protocol on the sensitivity and specificity of PCR for detection of naturally contaminated Salmonella in raw poultry compared to conventional culture. Food Microbiol. 23(6):599-604. Epub 2005 Nov 8.
- O'Regan E et al. (2008) Development of a real-time multiplex PCR assay for the detection of multiple Salmonella serotypes in chicken samples. BMC Microbiol. 21:8:156.
- Panigrahy B & Gilmore WC (1983) Systemic salmonellosis in an African gray parrot and salmonella osteomyelitis in canaries. J Am Vet Med Assoc. 15:183(6):699-700.
- Ward MP et al. (2003) Outbreak of salmonellosis in a zoologic collection of lorikeets and lories (Trichoglossus, Lorius, and Eos spp.). Avian Dis. 47(2):493-8.

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.