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FAST Avian Extraction Kit

REF **MBK0101** **48** **48 EXTRACTIONS**

INTRODUCTION AND PRODUCT DESCRIPTION

The FAST Avian Extraction Kit provides a procedure for DNA extraction from feathers for gender identification of monomorphic birds.
Lysis occurs by incubation at 56°C and vortex mixing in the Proteinase K, Dithiothreitol and Lysis Buffer specifically formulated to remove contaminants that could be found in bird feather samples. The presence of a resin which acts via ion exchange limits destruction of the DNA by inactivating nucleases and chelating heavy metals that may damage DNA.
The DNA extract is suitable for PCR detection using the FAST Avian Sexing PCR Kit (MBK0102, Diatheva).

KIT CONTENTS

- Lysis Buffer: 2X 28 mL
- Proteinase K: 1X 8.8 mg
- Dithiothreitol (DTT): 1X 126.5 mg
- Reconstitution Buffer: 1X 1.5 mL

ADDITIONAL EQUIPMENT AND MATERIAL REQUIRED

- Magnetic stir plate
- Water bath or thermomixer
- Sterile tweezers and scalpels
- Vortex
- Centrifuge for 1.5 mL tube for centrifugation at 13,000 rpm
- Powder free gloves
- Micropipettes and filter tips
- Sterile 1.5 ml vials

SHIPPING CONDITIONS

Shipping at room temperature has no detrimental effect on the performance of this kit

STORAGE

Upon arrival, store at +2 to 8°C. If stored at recommended temperature all reagents are stable until the expiration date.

GENERAL PRECAUTIONS

- The test must be performed by specialize and trained staff,
- Do not use reagents after the expiry date printed on the label,
- Use (powder free) gloves during the whole procedure,
- Change gloves often, especially if you suspect their possible contamination,
- After DNA extraction clean working space periodically with at least 5% sodium hypochlorite or other decontaminant agent,
- It is suggested to provide separate and dedicated spaces, material, and equipment for pre- and post-PCR amplification stages.

PROCEDURE

Notes Prior to Use:

- Reconstitute the vial of Proteinase K in 440 µL of Reconstitution Buffer, vortex until the powder is resuspended. Store the reconstituted solution at -20°C until needed
- Reconstitute the vial of Dithiothreitol (DTT) in 825 µL of Reconstitution Buffer, vortex until the powder is resuspended. Store the reconstituted solution at -20°C until needed
- Preheat a water bath or thermomixer to 56°C
- Prepare the number of 1.5 mL vials corresponding to the number of samples by pipetting 1 mL of Lysis Buffer in each vial using a 1000 µL micropipette

  Note: mix thoroughly the Lysis Buffer while pipetting the buffer into 1.5 mL vials on a magnetic stir plate (medium speed) in order to keep it in suspension and collect the resin (Lysis Buffer bottle contains a magnetic bar)

a) Cut the terminal portion of the feather (calamus) using sterile tweezers and scalpels (Fig.1) and clean it of all small feathers (Fig.2)

Fig.1: Cut of feather's terminal portion (calamus)

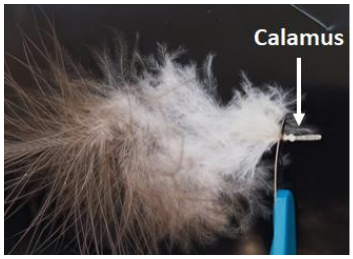

Fig.2: Clean calamus from small feathers

b) Put the calamus in a 1.5 mL tube with 1 mL Lysis Buffer previously prepared
c) Add to each sample 15 µL of Dithiothreitol and 8 µL of Proteinase K
   IMPORTANT NOTE: Ensure that the calamus is completely immersed in the buffer
d) Incubate in a thermomixer at 56°C for 30 min with a shaker speed of 1000 rpm
   The incubation can be done in a water bath at 56°C for 30 minutes vortexing the sample at maximum speed for 30 seconds at the start, after 15 minutes and at the end of the incubation

e) Centrifuge for 2 minutes at 13,000 rpm

NOTE: The sample is ready to test in PCR recovering the supernatant without pipette the pellet formed by the resin. The sample can be stored at -20°C for a limited period of time.