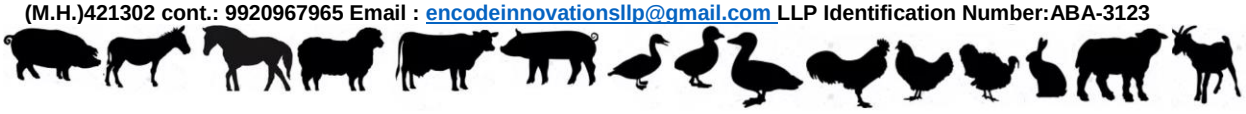
ENCODE CHICKEN IDENTIFICATION KIT

ENCODE INNOVATIONS LLP. B1- Ground Floor, Gayatri Res . Opp. Khandagle estate PURNA, Bhiwandi Thane (M.H.)421302 cont.: 9920967965 Email : encodeinnovationsllp@gmail.com LLP Identification Number:ABA-3123



ENCODE CHICKEN IDENTIFICATION KIT

ENCODE CHICKEN ID KIT Cat. No .EIL-CHICKEN-10
ENCODE CHICKEN ID KIT Cat. No .EIL- CHICKEN -25
ENCODE CHICKEN ID KIT Cat. No .EIL- CHICKEN -50
ENCODE CHICKEN ID KIT Cat. No .EIL- CHICKEN -100

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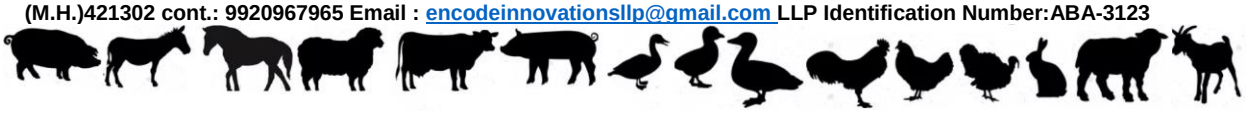
1. MEAT INFORMATION

In case of meat and meat products, the animal species must be disclosed on the label. Moreover, the species authenticity can be highly relevant to consumers for economic, medical, cultural and religious reasons. Fraudulent substitution of cheaper meats in place of more expensive species, the inclusion of meat in non-meat (vegetarian) products and the presence of allergens in food products are clear examples for the importance of this issue. Several methods for species detection are based on identification of proteins by means of electrophoretic and/or immunological methods. However, these methods are not reliable for application in highly processed and heated products due to the protein deterioration. DNA is more stable than proteins during processing and although it can be fragmented by several processes, modern DNA methodologies like PCR based techniques still allow the identification of DNA from the species present in a sample. Real time PCR techniques are especially suitable for these products because small fragments of DNA can still be amplified and identified with high sensitivity and specificity.

2. INTENDED USE

The kit enables a qualitative detection of chicken DNA in food samples by Real Time PCR, after a sample processing step. This DNA is present in the mitochondrial genome and it may be found in food and food industry. The test may be used with the following matrixes: chicken meat, poultry sausage, chicken burger, chicken nuggets.



3. STORAGE CONDITIONS AND STABILITY

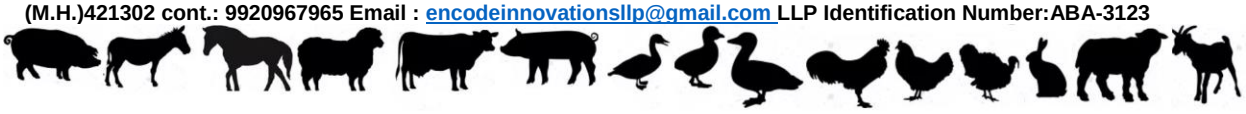
The kit must be stored at -20°C. The Master Mix and the Control should be stored between -20°C and 5°C. The kit must be shipped refrigerated, using coolers, and should be stored immediately on receipt. Repeated thawing and freezing should be avoided, as this may reduce the sensitivity. It should be protected from exposure to light. The kit is stable until the expiration date stated on the outer package and on the labels of the vials.

4. WARNINGS AND PRECAUTIONS

1. These products are exclusively for in vitro use.
2. Molecular Biology procedures, such as DNA extractions and PCR amplification, require qualified to prevent the risk of erroneous results, especially due to sample contamination or degradation of the nucleic acids contained in the sample.
3. It is strongly recommended to have dedicated areas, materials and equipment for the DNA extraction, preparation of the PCR and post-PCR procedures. Workflow in the laboratory should proceed in a unidirectional manner, from the Extraction Area to the Amplification and Detection Area.
4. The user should always pay attention to the following:
   - Read all the instructions provided with the product before running the assay.
   - Do not mix reagents from different batches.
   - Wear disposable gloves, laboratory coats and eye protection when handling specimens and reagents.
   - Avoid cross contamination of samples and reagent.
   - Use sterile pipette tips with filters.
   - Store and extract positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
   - Thaw all components thoroughly at room temperature before starting an assay. When thawed, mix the components and centrifuge.
   - Avoid contact of specimens and reagents with the skin, eyes and mucous membranes. If these solutions come into contact, rinse immediately with water and seek medical advice immediately.
   - Add the positive control after closing the all tubes.
5. Waste must be treated and disposed of in compliance with the appropriate safety standards.

5. ADDITIONAL MATERIALS REQUIRED

Disposable powder-free gloves
DNA extraction kit
Sterile pipette tips with filters
Tubes/Strips and accessories specific for the Real Time PCR Instrument



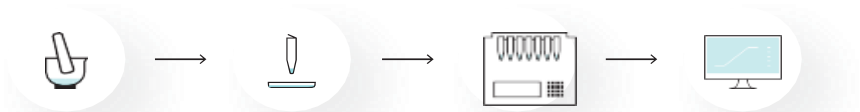
6. EQUIPMENTS REQUIRED

Laminar Air Flow Cabinets/PCR Cabinets
Micropipettes
Microcentrifuge
Real Time PCR instrument
accessories Freezer, refrigerator

7. SAMPLE COLLECTION AND TRANSPORT

This product must be used with DNA extracted from the following food samples: chicken meat, poultry sausage, chicken burger, chicken nuggets. Samples that are to be used for DNA extraction must be collected and stored according to laboratory guideline.

8.PROCESSING STEPS



Sample processing DNA Extraction PCR Set up and run Result and Interpretation

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Table 1:- The kit contains and Reaction Size available as below

Reagents	10 reactions	25 reactions	50 reactions	100 reactions
Master MIX	125 µl	312.5 µl	625 µl	1250 µl
Chicken detection mix	25µl	62.5µl	125µl	250 µl
PCR grade water	80µl	200 µl	400 µl	800 µl
Negative control	20 µl	50µl	100 µl	200 µl
Chicken Positive control	5 µl	10 µl	20 µl	40 µl

9.1. Experimental Protocol

Preparing the reaction.
Table 2:-

Reagent	Volume per reaction (25 µl)

Master mix	12.5 µl
Chicken Detection mix	2.5 µl
Sample /Template DNA	2 µl
PCR Grade water	8 µl
Total volume	25 µl

Preparing the PCR

1. Add 23µL of the chicken Reaction into each tube/strip.
2. Add 2µL of DNA sample.
3. Close the tubes/strips, and spin.
4. Place the reactions into the Real Time PCR instrument.

Program set up

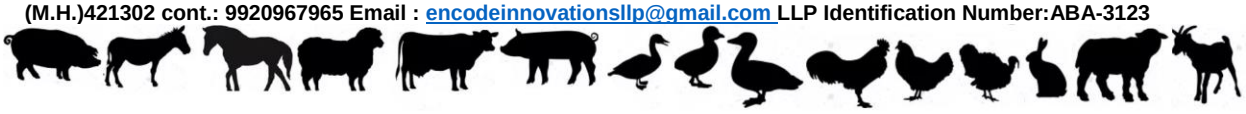
Stage	Temp	Time	No of cycles
Initial denaturation	95°c	10 min	1
Denaturation	95°c	15 5ec	
Annealing and Extension	60°c	60 sec	
Denaturing, Annealing and Extension			45

- Passive reference dye:-ROX

** If Needed, prepare a fresh dilution of ROX internal reference dye and use **0.3 µl** for each reaction. The diluted ROX reference dye must be kept in a light-protected tube at 4°c

- **For High ROX** compatible instrument dilute 5 µl ROX in 50 µl PCR Grade Water (1:10).
- **For Low ROX** compatible instrument dilute 0.5 µl ROX in 50 µl PCR Grade Water (1:100).

10. DATA ANALYSIS



Probe for Chicken detection are labeled with FAM and must be analyzed in the corresponding fluorescence channel. For each sample, compare the results obtained in each channel and interpret the results as described in the following tables:

A)Controls

To validate the assay, the controls must have the following results:

Negative control	Positive control	Interpretation
-	+	perfect
-	-	Rerun, pipetting error
+	+	Contamination, Rerun
+	-	Rerun, pipetting error

Note:- if the controls do not match these results, the experiment must be repeated

B)Samples

Interpretation of sample results is summarized in the following table:

Chicken detection FAM	IC (internal control)	Interpretation
+	+	Chicken detected(positive)
-	+	Chicken not detected(negative)
-	-	PCR inhibition , poor extraction
+	-	PCR inhibition, Rerun

**When both chicken and IC is not detected, the sample must be tested again after 1:10 dilution.

11. ADDITIONAL INFORMATION

For questions contact Email;- encodeinnovationsllp@gmail.com
Ms. Tithi patel-9970004798