SHANKARANARAYANA LIFE SCIENCES
A NEW GENERATION COMPANY

SN CHICKEN RT-PCR DETECTION KIT

Description of the Product:

The SN Chicken RT-PCR detection kit constitutes a ready to use system for multiple detection (presence or absence) using polymerase chain reactions (PCR) in the Real Time PCR machine. The Specific Master mix contains reagents and enzymes for the specific amplification of Chicken genes to generate amplified product and for the direct detection of the specific amplicon in fluorescence channel Cy5 of the Real Time PCR & the reference gene / Inhibition Control on HEX.

Intended to use:

RT-PCR based diagnostic kit for the quantitative detection of Chicken from a high level of food and feed safety, accurate animal species identification and the detection of adulterants are two of the greatest challenges facing food and feed products companies today.. It is recommended to use standard Tissue DNA extraction kit (spin column based or magnetic bead based) for isolation of Chicken DNA. The kit will also work on the automated DNA purification system. The kit intended to use by trained laboratory technician.

Contents of Kit:

Components	Color Code	Volume
RT Master Mix (MM)	Natural	1000µL
PP Mix	Amber Tube	200µL
Positive control (PC)	Blue	100µL
Nuclease free water (NC)	White	100µL

All Vials have Color Coder tops to distinguish between different reagents.

Storage:

The kit is shipped and stored at -200C

- **Sample which can be used for extraction:**

  Chicken (Tissue)

Procedure:

The performance of the SN Chicken RT-PCR Detection Kit depends on the amount and quality of template DNA purified from the tissue samples. The extraction protocol should be performed following manufacturer's instructions or an internally validated protocol.

SN PCR Master Mix preparation:

Prepare the master mix as per table mentioned below. Positive control (PC) and Negative test control (NC/NTC) should be included in every set up.

Color Code	Components	Volume (µL)/Reaction	Volume (µL) for 100 Reaction
Natural	SN Master Mix (MM)	10 µL	1000 µL
Amber Tube	PP Mix	2 µL	200 µL
Total Volume		12 µL	1200 µL

Add each component to multiple numbers of samples to be processed and prepare the master mix. It is advisable to prepare extra mix for one sample for any pipetting related error or dead volumes.

Note:

- Dispense 12 µL from above master mix in separate tubes/wells
- Add 4 µL of isolated DNA from sample and 4 µL of nuclease free water separately to each tube and total reaction mixture volume is 20 µL

Once dispensed, seal the plate with optically clear sealers or close the tubes-strips, centrifuge the tubes/strips or plates briefly and place them in the instrument.

Thermal Profile: (Setting of RT-PCR)

Referring to the instrument manual, set on the dedicated software for the parameters of thermal cycle.

Steps	Temp	Time	Cycle
Hold 1	**95 ℃**	**20 sec**	**1**
Cycling	**95 ℃**	**1 min**	**40**
	60 ℃	**20 secs**	
	72℃	**05 sec**	

Fluorescence Setup:

Applied Biosystem 7500	BioRad CFX-96	Rotor Gene Q
Cy5	**Cy5**	**Red Channel**
IC	**HEX**	**Green Channel**

Note:

The Kit is compatible with; Rotor Gene™ 3000/6000, ABI Step One, 7500/FAST, Quant studio, BioRad CFX 96, Roche 480 and other Real Time PCR machine available.

Precautions for PCR:

The following aspects should always be taken care of:

a) Store positive material (Positive controls & amplicons) separately from all other reagents and add it to the reaction mix in a separate area.

b) Thaw all components thoroughly at room temperature before starting the assay.

c) When thawed, mix the components and centrifuge briefly.

d) Work quickly on ice or in the Cooling Block.

e) All the reagents including the NTC (except for PCs & specimens) should be mixed & dispensed in pre-mix area.

f) All the controls & specimens should be mixed & dispensed in extraction area.

g) Use pipette tips with filters only.

h) Always use disposable powder-free gloves.

i) Discard samples and assay waste according to your local safety regulations.

j) Do not open the reaction tubes or plates post amplification.

Additionally, required Materials and Devices

a) Appropriate 3 or more channels RT-PCR instrument, compatible with the flurophores used in this test.
b) Appropriate nucleic acid extraction system or kit. It is recommended to use commercialized extraction kit extracting DNA.
c) Vortex mixer.
d) Centrifuge with a rotor for 2 mL micro centrifuge tubes.
e) Pipette tips with filters (disposable).
f) Powder-free gloves (disposable).
g) Class II Biological Safety Cabinet (BSC), ideally in a BSL-2 facility.

Result Interpretation:

Amplification of PC suggests absence of any inhibition during amplification. Amplification of IC in samples suggests proper DNA extraction. Amplification in Cy5 channels indicates presence of Chicken in sample. Due to competition and limiting factors, samples with high copy may not show amplification in HEX (IC) channel and the curves may not be a proper sigmoid. Results can be analyzed with the help as mentioned below:

Note:

Ct value in any sample /well >36 should be considered as below detection limit of the kit

Result Interpretation table:

S.NO	HEX (internal control)	Cy5	Result Interpretation
1.	+	+	Chicken tissue DNA detected
2.	+/-	-	Chicken tissue DNA Not detected

3.	+/-	+	Presumptive Chicken tissue DNA detected*
4.	+	-	Targets are valid, Chicken tissue DNA NOT detected
5	-	-	Invalid repeat sampling/extraction/amplification

***Retest the sample if the retest results are same, then it could be presumed as positive.

Analytical Performance:

Evaluation of the kit on the confirm positives and negative specimen showed 100% specificity and sensitivity. The kit can detect 1×10^3 copies per ml of Chicken DNA from tissue sample.

Trouble shooting:

Problem	Possible Cause	Recommendation
	Error in Master mix preparation.	Check the dispensing volume during preparation of master mixture
No signal in all samples including positive control	Inhibitors added Probe degradation Positive control degradation Omitted components Instrument setting error	Repeat the extraction step with new sample Use a new probe reagent Use a new positive control Verify each component, repeat the RT-PCR mixture preparation Check the position setting for the positive control on the instruments. Check the Thermal cycle settings on sample instrument
Diverse intensity of fluorescent signals	Pipetting error Contamination in the outer surface of PCR tubes or Plate	Make sure that the equal volume of reactants is added in each tube or plate Wear gloves during the experiment
Weak or no fluorescent signal in samples only	Poor DNA quality Insufficient volume of DNA	Use recommended kit for DNA extraction and store extracted DNA at -70°C Repeat PCR reaction with correct

		volume of DNA Fluorescence
No template Control(NTC) showing Amplification	Amplification of Chicken genes in a no Template Control indicates contamination in one or more of the reagents, incorrect placement of a plate or tubes into the RT-PCR instrument, or pipetting error	Repeat the test by using 2 NTC. If NTC fails again, then investigation should be conducted to identify possible causes of error

Technical Assistance:

For technical assistance please contact our technical Support:

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