

1. Description

VDx[®] Fowl Typhoid Typing PCR Kit is provides a range of testing for the detection of Salmonella enterica serovar Gallinarum biovars Gallinarum and Pullorum and the biovar Gallinarum live vaccine strain 9R and SR2-N6 by multiplex PCR method.

2. Storage

The components of VDx $^{\mbox{\scriptsize N}}$ Fowl Typhoid Typing PCR kit should be stored at -20°C, under this condition, the kit is stable until expiration date stated on the label.

3. Contents

Reagents	50 Tests
PCR Premix (dry form)	56T
Control DNA	100µl X 1
PCR grade water	1ml X 1
Instruction manual	1부

4. Template preparation

Briefly, several colonies of bacteria on agar plates, which originated from one single colony in the beginning, were picked with a sterile toothpick and inoculated into 100 μ of TE buffer in a microcentrifuge tube. The cell suspension was boiled in a water bath for 5 min and then briefly centrifuged to pellet cell debris. The supernatant was transferred to a new tube and used as the PCR template.

5. PCR method

- 1) Prepare appropriate PCR Premix tubes.
- 2) Add $19\mu\ell$ of PCR grade water into PCR premix tube.
- 3) Add $1\mu\ell$ of template DNA into the PCR premix tube.
- 4) Gently mixed and briefly centrifuged .
- 5) Perform PCR reaction of samples as the below process using PCR machine.

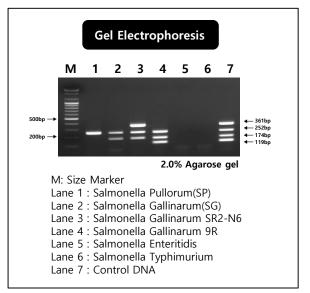
Step	PCR Cycle			
	Temp.	Time	Cycle	
Initial Denaturation	94°C	5 min	1 cycle	
Denaturation	94°C	30 sec	30 cycles	
Annealing	64°C	30 sec		
Extension	72℃	60 sec		
Final Extension	72℃	7 min	1 cycle	

6. Detection of Amplified Products

- 1) Prepare 2.0% agarose gel containing Ethidium bromide (Et-Br).
- 2) Load 5 μ of PCR product on agarose gel without adding a loading dye buffer and perform electrophoresis.
- 3) Run electrophoresis by 100V(required about 30~40min).
- 4) Identify the result on ultra-violet(UV) transilluminator.

7. Interpretation

Pathogen	Band size				
SP	-	-	252bp	-	
SG	-	174bp	252bp	-	
SG-9R	119bp	174bp	252bp	-	
SG-SR2-N6	-	174bp	252bp	361bp	
Control DNA	119bp	174bp	252bp	361bp	



8. Notice

- For research purpose only. Not for use in diagnostic procedures for clinical purposes. *For in Vitro Use Only.*

- Do not use any reagent after the expiration date.
- Do not use together with reagents of other products.

9. Trouble shooting

- In the case of difficult to interpret results due to non-specific bands
 Reduce amount of template by 1/10 dilution and reacts again.
- 2) Preparation of PCR reaction at room temperature may cause the non-specific band.
- 3) All procedure should be carried out on ice.