Appendix (2)

Table (1): Real time PCR reaction mix components

Component	**Volume/reaction**
QuantiNova SYBR Green PCR Master Mix	10 µl
QuantiTect Primer Assay	2 µl
RNase-Free water	3 µl
Total volume	**15 µl**

The reaction mix was mixed thoroughly and 15 µl was dispensed into each PCR strip tube. Five µl of the template cDNA was added to the PCR strip tube containing the reaction mix. Then, the Rotor-Gene Q **(Qiagen, Valencia, CA, USA)** was programmed according to table (2).

Table (2): Real-time cycler conditions

Step		**Time**	**Temperature**
PCR initial activation step		2 min	95ºC
2-step cycling	**Denaturation**	5 s	95ºC
	Combined annealing/ extension	10 s	60ºC
	Number of cycles: 40 cycles		

The PCR strip tubes were placed in the Rotor-Gene Q and the cycling program was started. Data acquisition was performed during the combined annealing/ extension.