Annex C
(informative)

Real-time RT-PCR mastermixes and cycling parameters

For the composition of one-step, real-time RT-PCR mastermixes using the Invitrogen RNA UltraSense™ one-step qRT-PCR system, see Table C.1. For cycling parameters, see Table C.2.

Table C.1 — Mastermix

Reagent	Final concentration (in 25 µl reaction volume)	Volume per reaction (µl)
5× UltraSense reaction mix	1×	5 ± 0,25
FW Primer	0,5 pmol/µl	as required
REV Primer	0,9 pmol/µl	as required
Probe	0,25 pmol/µl	as required
ROX reference dye (50×)	as required[a]	as required
RNA UltraSense enzyme mix	—	1,25 ± 0,1
Water (5.2.1)	—	as required
Total volume	—	20 ± 0,5

[a] With Applied Biosystems™ real-time PCR machines, ROX shall be used at 1× concentration; for the Stratagene MX3000™, ROX can be either used at 0,1× concentration, or omitted from the mastermix. For other machines, consult the manufacturer's instructions.

NOTE Applied Biosystems real-time PCR machines and the Stratagene MX3000 are products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the products named.

Table C.2 — Cycling parameters

Step description		Temperature and time	Number of cycles
RT		55 °C for 1 h	1
Preheating		95 °C for 5 min	1
Amplification	Denaturation	95 °C for 15 s	45
	Annealing-extension	60 °C for 1 min	
		65 °C for 1 min	

1) Invitrogen RNA UltraSense™ is the trademark of a product supplied by Invitrogen. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.