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PROBESURE™ ONESTEP RT-PCR MASTER MIX

USER GUIDE

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CONTENT

1. PRODUCT DETAILS

PRODUCT	PRODUCT VOLUME	PART NUMBER	REACTIONS AT 10 µL	CONCENTRATION
ProbeSure™ OneStep RT-PCR Master Mix (**No ROX**)	1 mL	RT-PSURE-01	200	2x
	10 mL	RT-PSURE-02	2,000	2x
	25 mL	RT-PSURE-03	5,000	2x
	250 mL	RT-PSURE-04	40,000	2x
ProbeSure™ OneStep RT-PCR Master Mix (**Low ROX** – 25 nM)	1 mL	RT-PSURE-05	200	2x
	10 mL	RT-PSURE-06	2,000	2x
	25 mL	RT-PSURE-07	5,000	2x
	250 mL	RT-PSURE-08	40,000	2x
ProbeSure™ OneStep RT-PCR Master Mix (**High ROX** – 500 nM)	1 mL	RT-PSURE-09	200	2x
	10 mL	RT-PSURE-10	2,000	2x
	25 mL	RT-PSURE-11	5,000	2x
	250 mL	RT-PSURE-12	40,000	2x

2. DESCRIPTION

ProbeSure OneStep RT-PCR Master Mix is designed for highly specific and sensitive one-step reverse transcriptase (RT) fluorescently-reporting PCR using RNA template. The master mix includes optimised components which allow reverse transcription and subsequent PCR amplification to take place in the same reaction well. ProbeSure OneStep RT-PCR Master Mix may be used in endpoint or real-time modes.

ProbeSure OneStep RT-PCR Master Mix is designed for use in PCR genotyping applications with hydrolysis probe-based assays. It can be used with a variety of fluorogenic probe types including TaqMan™, ZEN™ probes and BHQ™/BHQ*plus*™ probes. It is not intended for use as a general PCR master mix or for HRM applications.

ProbeSure OneStep RT-PCR Master Mix contains all the necessary components to carry out the reaction (except RNA and primers/probes) and is supplied at 2x concentration. It is formulated with dTTP as this improves reaction sensitivity and efficiency when compared to mixes containing dUTP.

3. STORAGE AND SHELF LIFE

ProbeSure OneStep RT-PCR Master Mix is shipped on blue ice. Upon arrival, store at -20˚C/-80˚C (stable for two years); multiple freeze / thaw cycles are not recommended. The mix can also be stored at 4˚C for two weeks (protected from light).

4. SAFETY WARNINGS AND PRECAUTIONS

This product should only be handled by trained laboratory personnel. It is advisable to wear suitable personal protective equipment (PPE) when using the product. In case of contact with skin or eyes, wash immediately with water.

5. KIT COMPONENTS

ProbeSure OneStep RT-PCR Master Mix (supplied at 2x concentration), containing a specifically engineered Taq polymerase, dNTPs, buffer, performance enhancers, $MgCl_2$, passive reference dye (ROX) and Reverse Transcriptase (RT) (200x).

REQUIRED COMPONENTS

- Fluorescent plate reader or qPCR machine capable of reading the fluorophores used
- PCR plate or equivalent and appropriate optically clear seal
- Template RNA
- PCR-grade water
- Primers and fluorogenic hydrolysis probes (or both combined into an assay)

6. ROX COMPATIBILITY

ProbeSure OneStep RT-PCR Master Mix is supplied without ROX, or with low or high ROX levels. Please ensure compatibility between the ROX level of the master mix and the qPCR instrument. Should you require further assistance, please contact your qPCR instrument/plate reader manufacturer, or contact 3CR Bioscience's Technical Support team.

If a fluorescent plate reader is used instead of a qPCR machine, it is recommended that the high ROX version of the ProbeSure OneStep RT-PCR Master Mix is used.

7. PRIMER AND PROBES

The optimal primer and fluorogenic hydrolysis probe concentrations should be determined empirically. Primer concentrations of 300 - 900 nM and probe concentrations of 100 - 200 nM are generally suitable for most applications.

8. RNA QUALITY AND QUANTITY

RNA is highly susceptible to ubiquitous RNases. Care should be taken when handling the samples. An empirical optimisation of RNA concentration should be carried out by testing a sample dilution range.

9. CONTROLS

To improve confidence in the data, control samples should be used on the PCR plate in addition to the test samples. Negative controls (no-template controls, or NTCs) should always be used and consist of the same

buffer used to hydrate the RNA samples, dispensed into several wells of the PCR plate. Positive controls can also be used, if available, and should consist of RNA samples of known sequence.

When viewing the data, NTCs should show no amplification, giving confidence that any amplification observed is real. Any amplification observed in the NTC wells would indicate contamination or non-specific amplification. The positive control samples should amplify as expected for their sequence.

10. REACTION PROCEDURE

A. ARRAYING TEMPLATE RNA

A liquid-handling system appropriate to the number of RNA samples to array should be used. RNA samples can also be arrayed manually if working with a low number of samples.

B. REACTION ASSEMBLY

Ensure the components are defrosted thoroughly, mix and centrifuge briefly prior to use. The enzymes used in ProbeSure OneStep RT-PCR Master Mix are modified such that they are completely inactive at ambient temperature. Such inactivation allows bench top reaction assembly without leading to primer-dimer issues and other side reactions. The enzymes are reactivated during the initial stage of thermal cycling.

COMPONENT	FINAL CONCENTRATION	VOLUME FOR 10 µL REACTION (µL)
ProbeSure OneStep RT-PCR Master Mix (2x)	1x	5
Primer(s)	300-900 nM	Variable
Probe(s)	100-200 nM	Variable
RNA template	Variable	Variable
Nuclease-free water	-	To 10 µL
TOTAL	**-**	**10 µL**

Table 1. *Reagent volumes for total reaction mix.*

A total reaction mix should always be made to eliminate well-to-well variation of component concentrations. Assemble the total reaction mix using *Table 1* as a guide.

ProbeSure OneStep RT-PCR Master Mix can be used in reaction volumes of any size, including volumes (below 1 µL) in the appropriate PCR plate. It is important that ProbeSure OneStep RT-PCR Master Mix is used at a final concentration of 1x.

C. TOTAL REACTION MIX DISPENSING & PLATE SEALING

The total reaction mix must now be dispensed into the PCR plate wells. As with DNA dispensing, use a liquid-handling system that is appropriate to the scale of the work. Once the total reaction mix has been dispensed, the PCR plate must be sealed with an optically clear seal and centrifuged to ensure all components are at the bottom of the wells.

ProbeSure OneStep RT-PCR Master Mix can be used with any standard Peltier-based or water-bath thermal cycler. If used in real-time mode, any appropriate qPCR instrument may be used, and the fluorescent data captured at each cycle. Use the thermal cycling protocol detailed in *Table 2*.

D. THERMAL CYCLING

Place the plate on a thermal cycler or qPCR instrument and carry out the thermal cycling step. The thermal cycling protocol used will vary with probe type but a guide to appropriate conditions is shown in *Table 2*.

STEP	DESCRIPTION	TEMP.	TIME	NO. CYCLES
1	Reverse transcription	50˚C	10-30min	1
2	Enzyme activation	95˚C	10-15 min	1
3	Template denaturation	95˚C	10-15 secs	35-45
	Annealing and extension	57-65˚C	60 secs	

Table 2. Thermal cycling guide for ProbeSure OneStep RT-PCR Master Mix reactions.

DESCRIPTION	TEMPERATURE	TIME	CYCLES PER STEP
Template denaturing	94˚C	10-15 secs	3
Annealing and extension	57-65˚C	60 secs	

Table 3. Thermal cycling conditions for recycling ProbeSure OneStep RT-PCR Master Mix reactions

E. FLUORESCENT SIGNAL DETECTION

After thermal cycling is complete, the fluorescent signal is detected and assessed by reading the plate with a fluorescent plate reader or a qPCR instrument in endpoint or real-time modes. If sufficiently defined genotype clusters are not obtained after the initial thermal cycling protocol, the plate should be cycled for an additional three cycles using the conditions detailed in *Table 3* and read/analysed again. The additional cycling can be repeated until tight and well separated clusters are observed, though this is rarely required.

If analysing in real-time, data acquisition should be carried out detected during the annealing/extension step of each cycle.

F. INTERPRETATION OF DATA

Endpoint data analysis and interpretation can be done using cluster analysis software, a qPCR instrument or alternatively can be carried out in Microsoft Excel.

ROX passive reference dye can also be used to eliminate the effect of well-to-well liquid volume differences from the resultant cluster plot data. The inclusion of a passive reference leads to tighter clustering and, as a result, more accurate scoring of data.

Real-time data analysis is carried out using the qPCR instrument software.

11. ORDERING INFORMATION

For ordering details, please visit www.3crbio.com

12. SUPPORT

If you require any support with the use of ProbeSure OneStep RT-PCR Master Mix or other 3CR Bioscience products, please contact our Technical Support team on support@3crbio.com.

13. LEGAL INFORMATION

For Research Use Only. Not for use in diagnostic procedures.

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