MAKING SCIENCE AFFORDABLE

PROBESURE™ MULTIPLEX MASTER MIX

USER GUIDE

telephone: +44 (0)1279 940 983
fax: +44 (0)1707 240 451
email: support@3crbio.com
web: https://3crbio.com/

Unit 10, West Point Business Park, West Road, Harlow, Essex, CM20 2BU United Kingdom

CONTENT

1. PRODUCT DETAILS

PRODUCT	PRODUCT VOLUME	PART NUMBER	NUMBER OF REACTIONS AT 10 µL	CONCENTRATION
ProbeSure™ Multiplex Master Mix (**No reference dye**)	2.5 mL	006-0001	500	2x
	10 mL	006-0002	2,000	2x
	25 mL	006-0003	5,000	2x
	200 mL	006-0004	40,000	2x
	1,000 mL	006-0005	200,000	2x
ProbeSure™ Multiplex Master Mix (**with ATTO 633**)	2.5 mL	006-0006	500	2x
	10 mL	006-0007	2,000	2x
	25 mL	006-0008	5,000	2x
	200 mL	006-0009	40,000	2x
	1,000 mL	006-00010	200,000	2x

2. DESCRIPTION

ProbeSure Multiplex Master Mix is designed for use in PCR-genotyping applications with hydrolysis probe-based assays, using either endpoint or real-time detection. It is formulated for detection of Single Nucleotide Polymorphisms (SNPs) and insertion/deletions (Indels), or detection of sequences of interest.

ProbeSure Multiplex Master Mix can be used with a variety of fluorogenic probe chemistries 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 Multiplex Master Mix contains all the necessary components to carry out the reaction (except DNA 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 Multiplex 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. ProbeSure Multiplex Master Mix can be aliquoted into light-protective tubes to reduce the need for repeated freeze-thaw cycles. 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 Multiplex Master Mix (2x concentration), containing a specifically engineered Taq polymerase, dNTPs, buffer, performance enhancers and $MgCl_2$.

REQUIRED COMPONENTS

- Fluorescent plate reader or qPCR instrument capable of reading the fluorophores used in the intended assay
- PCR plate or equivalent and appropriate optically clear seal
- PCR-grade water
- Primers and probes (or both combined into an assay)
- Template DNA.

6. NORMALISING DYE

ProbeSure Multiplex Master Mix is supplied either with or without ATTO 633 normalising dye.

7. PRIMERS AND PROBES

The optimal primer and 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. DNA QUALITY AND QUANTITY

It is recommended to use 1-10 ng of gDNA per reaction well, though this will vary with organism genome size (large genomes will require a proportionately larger DNA mass). For optimal results, purified and well-normalised DNA samples should be used. However, when using ProbeSure Multiplex Master Mix in high throughput, purified DNA is often not commercially practical. ProbeSure Multiplex Master Mix contains inhibitor resistant components and so will generally work well with DNA that has been crudely extracted, but such samples should be tested before commencing large-scale work. Empirical optimisation of DNA concentration by testing a sample dilution range test is the most sensible approach.

9. CONTROLS

To have confidence in the genotyping data, control samples should be used on the PCR plate in addition to the test samples. Negative controls (no-template controls, NTCs) should always be used and consist simply of the same buffer used to hydrate the DNA samples, dispensed into several wells of the PCR plate. Positive controls can also be used if available and should be DNA samples of known genotype.

When viewing genotyping data, NTCs should show no amplification and remain at the origin of the cluster plot (see *Figure 1*), giving confidence that any amplification observed is true. Any amplification observed in the NTC wells would indicate contamination or non-specific amplification. The positive control samples should cluster in the expected regions for their genotype.

10. REACTION PROCEDURE

A. ARRAYING TEMPLATE DNA

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

ProbeSure Multiplex Master Mix can be used with hydrated or dry DNA samples. Both approaches work equally well but have practical advantages and disadvantages. If low numbers of samples are to be genotyped, it is not worth drying the DNA samples.

However, if high numbers of samples are to be genotyped in one run, drying the DNA samples can improve the resulting genotyping data. Hydrated DNA arrayed in a PCR plate will quickly begin to evaporate differentially across the plate (samples near the edges evaporate more quickly than those in the middle). Variation in DNA volumes across the plate will lead to variation in the final reaction concentrations, causing sub-optimal genotyping results. For this reason, when drying the DNA into the plate wells the user must ensure that the DNA has been dried to completion. Dried DNA samples will be stable long term at ambient temperature.

If a very small reaction volume is to be used (for example 1.0 µL total volume), it might not be possible to accurately dispense 0.5 µL of DNA and 0.5 µL of total multiplex reaction mix. In this example, drying the sample would allow a more realistic 1.0 µL of total reaction mix to be dispensed to the well.

To dry the DNA, once dispensed into a PCR plate, the plate should be centrifuged to ensure the samples are in the bottom of the wells and then placed in a laboratory fan oven for one hour at around 55°C, or until the samples have visibly dried. When assembling the total multiplex reaction mix, water must be added in the correct proportion to account for the missing volume of the DNA template.

B. REACTION ASSEMBLY

Ensure the components are defrosted thoroughly, mix and centrifuge briefly prior to use. The enzymes used in ProbeSure Multiplex 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 Multiplex MM (2x)	1x	5
Primer(s)	300-900 nM	Variable
Probes	100-200 nM	Variable
DNA template[1]	Variable	Variable
Nuclease-free water	-	To 10 µL
Total	**-**	**10 µL**

Table 1. Reagent volumes for total multiplex reaction mix.

[1]Final concentration of cDNA 0.1 pg/µL; gDNA 10 pg/µL - 10 ng/µL

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

ProbeSure Multiplex 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 Multiplex Master Mix is used at a final concentration of 1x.

C. TOTAL MULTIPLEX REACTION MIX DISPENSING & PLATE SEALING

The total multiplex 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 multiplex 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.

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	Enzyme activation	95˚C	10-15 min	1
2	Template denaturation	95˚C	10-15 secs	35-45
	Annealing and extension	57-65˚C	60 secs	

Table 2. Thermal cycling guide for total multiplex reactions

E. FLUORESCENT SIGNAL DETECTION

After thermal cycling is complete, the fluorescent signal is detected by reading the plate in an appropriate fluorescent plate reader or qPCR machine in endpoint mode.

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.

DESCRIPTION	TEMP.	TIME	NO. CYCLES
Template denaturing	94˚C	10-15 secs	3
Annealing and extension	57-65˚C	60 secs	

Table 3. Thermal cycling conditions for recycling ProbeSure Multiplex Master Mix reactions.

F. INTERPRETATION OF DATA

Endpoint data analysis and interpretation can be carried out using cluster analysis software or alternatively can be carried out in Microsoft Excel (see *Figure 1a*).

ATTO 633 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.

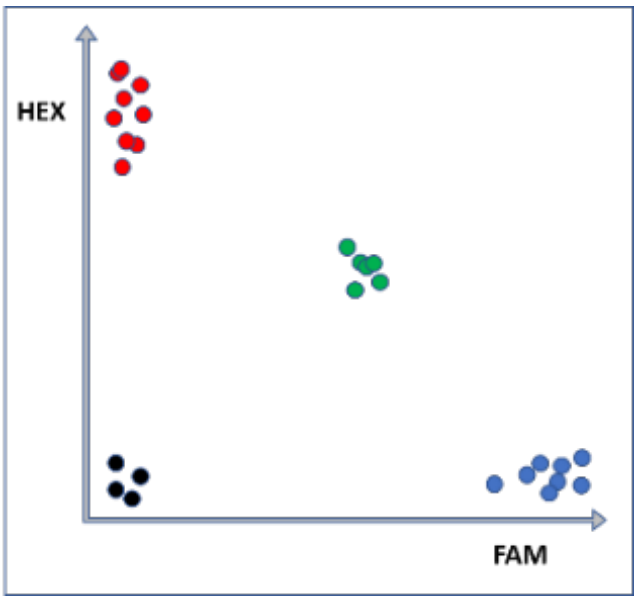

Figure 1a. Diagram of typical genotyping cluster plot data generated using ProbeSure Multiplex Master Mix (FAM and HEX data shown). Black samples at the origin are the no-template controls (NTCs).

Depending on the application, data can be collected in real-time and viewed as in the example shown in *Figure 1b.*

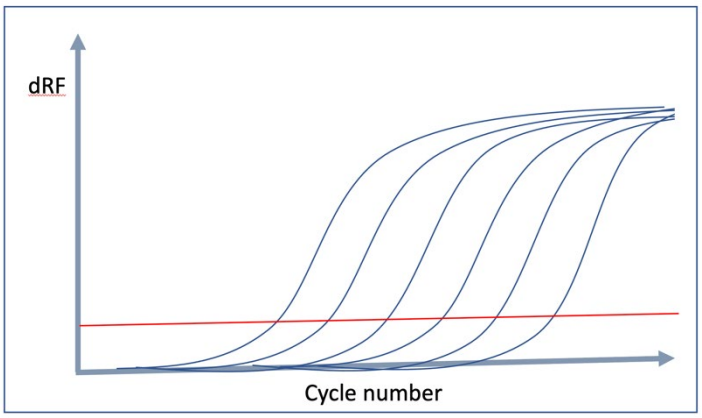

Figure 1b. Diagram of example real-time serial dilution plot generated using ProbeSure Multiplex Master Mix.

11. ORDERING INFORMATION

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

12. SUPPORT

If you require any support with the use of ProbeSure Multiplex 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.

3CR Bioscience disclaims all warranties with respect to this documentation.

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly or by implication. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration.

TaqMan™ is a trademark of Roche Molecular Systems, Inc.

Zen™ Probes is a trademark of Integrated DNA Technologies, Inc.

BHQ™ Probes and BHQplus™ Probes are trademarks of LGC Biosearch Technologies