CIRRUS™ RNA Master Mix
INSTRUCTIONS FOR USE

FLUOROGENICS
Polymerase Chain Reaction Specialists

INTRODUCTION

Cirrus™ (Product code CIR-RNA-1000-01) is a 'ready-to-use' pre-formulated master mix that is specifically designed for fluorogenic probe based reverse transcription Polymerase Chain Reaction (RT-PCR) applications using the 5' nuclease signalling principle and RNA/DNA targets. It is also suitable for other dual labelled fluorogenic probe methods based on conformational signalling. **Cirrus™** has been formulated using a proprietary blend of sugars, stabilisers and macromolecules in a freeze drying process providing instant reagent dissolution using only water. This drying process ensures the entire active components remain stable at ambient temperatures and eliminates the requirement for refrigerated reagent transport and storage. Once opened the **Cirrus™** cake is resistant to high humidity levels for up to one hour making it ideal for automation.

Cirrus™ contains the core reaction components required to carry out a RT-PCR, this includes: core PCR reaction buffer, dNTP (inc dUTP) nucleotides, Magnesium ions and a high performance rapidly activated HotStart *Taq* DNA polymerase and a thermostable *MMuLV* RNA dependant DNA polymerase. This enzyme provides superior conversion of RNA to cDNA at elevated temperatures, increasing primer stringency and reducing RNA secondary structure. Supplied as a dry reagent, **Cirrus™** may be reconstituted using water and the addition of target-specific oligonucleotide primers and probes (not supplied) to provide a complete RT-PCR reaction mix.

Highly flexible, the **Cirrus™** reagent is readily compatible with automated laboratory dispensing systems and may be reconstituted to suit a variety of end-user test requirements. This includes the addition of other buffer solutes and third party enhancers such as Uracil-N-glycosylase (UNG).

Cirrus™ may be reconstituted in a volume that allows more template or reagents to be added than conventional 2x frozen mixes. This may be useful when using multiple primer sets in a multiplex format, or where sensitivity is needed (more template). Each vial contains an ~7.5% reagent excess and should be reconstituted to the following volumes:

Master Mix concentration req'd	Diluent Volume
10X	**135 µL**
5X	**270 µL**
2X	***675 µL****

Typical application

Example protocols are provided below using **Cirrus™** reagents. Given the unique versatility of **Cirrus™**, many experimental protocols are possible and those provided are intended as a guideline only.

PROTOCOLS

Opening of **Cirrus™** Vial: Remove the cap. Gently tap the glass vial to settle contents that may have shifted during transit. Remove neoprene stopper and resuspend as described in the following user protocols and vortex mix briefly.

Protocol 1: 50 x 25 µL PCR reactions using 5µl template per reaction.

The **Cirrus™** vial contents may be reconstituted to a volume of 675 µL to provide a 2X core reaction mix. This is then used to produce a sub master containing primers and probes. This is sufficient mix for 50 x 25 µL (final) PCR reactions as follows:

Reagent	Volume
Cirrus™ ***vial*** *(Product code CIR-RNA-1000-01) Resuspended to 2 x concentration using sterile water (*675 µL*). Withdraw 625 uL.*	625 µL
Oligonucleotide Primers *(not supplied)* Forward Primer to a final reaction concentration of 0.1 to 1µM Reverse Primer to final reaction concentration of 0.1 to 1 µM	e.g. 12.5-125 µL 10µM stock solution e.g. 12.5-125 µL 10µM stock solution
Oligonucleotide Probe *(not supplied)* Probe (Dual labelled hydrolysis probe / Molecular Beacon) to a final reaction concentration 0.05 to 0.2µM	e.g. 31.25-125 µL of 2µM stock
Diluent/ Nuclease-free water	To final Volume
TOTAL	**1000 µL**

The reaction mix is then aliquotted (50) in 20 µL volumes in PCR reaction tubes and mixed with 5µL nucleic acid template ready for thermocycling. No template controls may use water to substitute template RNA or DNA.

Thermal Cycling: The following are general recommended thermal cycler settings for **Cirrus™** reagents. Actual hold temperatures, times and transition rates may vary according to PCR assay type and instrument used.

Phase	Hold	Temp (°C)	Time (s)	Rate (°C/s)
Reverse Transcription	Hold	40-55	60-120	3-10
Enzyme Activation	Hold	95	60	3-10
Amplification	Denature	95	5-10	3-10
	Anneal & Extend	50-65	5-30	3-10

Protocol 2: 50 x 25 µL RT-PCR reactions using 12.5µl template per reaction.

The **Cirrus™** vial contents may be reconstituted to a volume of 270 µL to provide a 5X core reaction mix. This is then used to produce a 2x sub master containing primers and probes which can be added to template in equal volume. This is sufficient mix for 50 x 25 µL (final) PCR reactions as follows:

Reagent	Volume
Cirrus™ ***vial*** *(Product code CIR-RNA-1000-01) Resuspended to 5 x concentration using sterile water (270 µL). Withdraw 250 uL.*	250 µL
Oligonucleotide Primers *(not supplied)* Forward Primer to a final reaction concentration of 0.1 to 1µM Reverse Primer to final reaction concentration of 0.1 to 1 µM	e.g. 12.5-125 µL 10µM stock solution e.g. 12.5-125 µL 10µM stock solution
Oligonucleotide Probe *(not supplied)* Probe (Dual labelled hydrolysis probe / Molecular Beacon) to a final reaction concentration 0.05 to 0.2µM	e.g. 31.25-125 µL of 2µM stock
Diluent/ Nuclease-free water	To 625 µL final Volume
TOTAL (for 2X reaction mix)	**625 µL**

The 2X reaction mix is then aliquotted (50) in 12.5 µL volumes in PCR reaction tubes and supplemented with 12.5µL of RNA or DNA template ready for thermocycling. No template controls may use water to substitute template RNA or DNA.

Thermal Cycling: As per protocol 1

ADDITIONAL INFORMATION

Cirrus™ reagent is formulated to provide 5mM final reaction Magnesium ions. This is optimum for most 5' nuclease assays. This concentration can be increased to suit the requirements of different assays by substituting diluent with additional $MgCl_2$ solution (not supplied) in the final master mix.

Cirrus™ reagent is formulated to provide a base performance. This formulation can be adjusted to suit the requirements of different assays/ PCR instruments by substituting diluent with many additional reaction adjuncts.

STORAGE

Cirrus™ mastermix is supplied as a dried reagent and should be stored in its original packaging at 15-30°C. Once reconstituted, the **Cirrus™** mastermix will remain stable for 24 hours if stored at 2-8°C. For longer term storage as a conventional mastermix reagent, **Cirrus™** mastermix should be resuspended to 2x concentration with a diluent supplemented with 8-16% (v/v) molecular biology grade glycerol and stored at -20°C. Use within 6 months.

TECHNICAL SPECIFICATION

Specification	Dimension
DNA dependant DNA Polymerase	*High Performance Hot Start Taq* polymerase (derived from *Thermus aquaticus*)
RNA dependant DNA Polymerase	*High performance thermostable RNase H+ recombinant MMuLV*
Nucleotides	dNTP containing proprietary dUTP mix
Buffer	Tris, pH 8.8 5' nuclease assay specific salts & enhancers
Magnesium Chloride	5 mM May be increased by user
Storage	15 to 30 °C
Shelf life	18 Months from manufacture date
Dissolution time	< 1s
Volume (final) upon dissolution	User defined >135µL. (Final reaction volume 1350µL)
BSA	Contains Bovine Serum Albumin of USA origin certified BSE free.

MANUFACTURER DETAILS

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