Cirrus™ Strips-RNA RT-PCR Master Mix
INSTRUCTIONS FOR USE

INTRODUCTION

Cirrus™ Strips RNA (Product code CIR-S-RNA-01) is a 'ready-to-use' pre-dispensed (96-wells) dry master mix that may be used for RNA amplification and detection using the 5' nuclease real-time RT-PCR method. **Cirrus™ Strips RNA** has been formulated using Fluorogenics Cirrus™ reagent, a proprietary blend of sugars, stabilizers and macromolecules produced in a freeze drying process providing instant reagent dissolution. This drying process ensures the entire active components remain stable at ambient temperatures and eliminates the requirement for refrigerated reagent transport and storage.

Cirrus™ Strips RNA contains the core reaction components required to carry out a 5' nuclease RT-PCR, this includes: core RT-PCR reaction buffer, dNTP nucleotides, Magnesium ions, a thermostable *MMuLV* reverse transcriptase polymerase, and a high performance *Taq* DNA polymerase exhibiting 5' to 3' exonuclease activity. Supplied as a dry reagent, each well of **Cirrus™ Strips RNA** is reconstituted using target-specific oligonucleotide primers and probes (not supplied) to provide a complete 20 µL RT-PCR reaction mix within each well of the plate.

Cirrus™ Strips RNA is provided in a generic low profile 96-well fully skirted PCR plate that may be used directly on a wide variety of commercial cyclers. Alternatively, 8 well strips may be removed and used individually. Using an alternative frame Cirrus™ Strips RNA can be configured for ABI FAST Block or Roche LightCycler 480. The guidance platform compatibility is provided below.

PROTOCOLS

1. Open the **Cirrus™ Strips RNA** Mylar® foil packet by tearing along the pre-cut tear notch and remove the 96-well plate.

2. Gently tap the plate to settle its contents to the bottom of each well.

3. Remove the temporary adhesive seals from wells and add reagents as described in the user protocol described below. It is recommended that primer and probes are prepared as a sub- mix before dispensing into each plate well to minimise volumetric errors. The **Cirrus™ Strips RNA** reagent undergoes almost instant dissolution and does not require mixing - the reaction is sufficiently mixed by convection alone (during the PCR thermocycling enzyme activation phase).

4. The supplied optical lids can be applied to each strip or the whole plate once dispensing is complete. Alternatively, a new permanent PCR seal may be applied to the plate (not supplied). The plate may be centrifuged to collect the contents to the bottom of each well should any reaction mix have collected on the well walls following dispensing. Alternatively tapping the plate, once sealed, onto a flat surface should allow the reaction to collect at the apex of each tube.

Protocol 1: 20µL Probe-based RT-PCR reactions using 10µl template per reaction.

Each **Cirrus™ Strips RNA** contents may be reconstituted to a final volume of 20µL to provide a RT-PCR reaction as follows:

Reagent	Volume
Cirrus™ Strips RNA *(Product code* CIR-S-RNA-1000-01*)*	-
Oligonucleotide Primer *(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. 2µL 10µM stock solution e.g. 2µL 10µM stock solution
Oligonucleotide Probe *(not supplied)* Probe (Dual labelled 5' nuclease probe) to a final reaction concentration 0.05 to 0.2µM	 e.g. 2µL of 2µM stock
Diluent/ Nuclease-free water	e.g. 4µL
TOTAL (2X RT-PCR reaction mix)	**10 µL**
Template (water for NTC)	**10 µL**
TOTAL (1X RT-PCR reaction mix)	**20 µL**

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

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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

ADDITIONAL INFORMATION

Cirrus™ Strips RNA reagent is formulated to provide 5mM final reaction Magnesium ions. This concentration can be adjusted to suit the requirements of different assays (above 5 mM) by substituting diluent with additional $MgCl_2$ solution (not supplied) in the final master mix.

STORAGE

Cirrus™ Strips RNA is supplied as a dried reagent and should be stored in its original packaging at 15-30°C for up to 18 months. If opened but not used immediately, the plate may be re-sealed in its original zip-lock Mylar® pouch but should be used within 72 hours when stored with the desiccant provided.

DISPOSAL

Dispose of the plate and packaging as laboratory waste according to local rules.

TECHNICAL SPECIFICATION

Specification	Dimension
RNA dependant DNA Polymerase	*High performance thermostable RNase H+ recombinant MMuLV*
DNA dependant DNA Polymerase	High Performance full length *Taq* polymerase (derived from *Thermus aquaticus*)
Nucleotides	dNTP with dUTP
Buffer	Tris-HCl, pH 8.8
Magnesium Chloride	5 mM May be adjusted (increased) by user
Storage	15 to 30 °C
Shelf life	24 Months from manufacture date
Dissolution time	< 1s
BSA	Contains Bovine Serum Albumin of Canadian origin.

MANUFACTURER DETAILS

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DISCLAIMER

For Research Use Only, this product may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Fluorogenics products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of Fluorogenics Ltd.

Cirrus™Strips PCR Platform Compatibility

With semi-skirted Roche consumable accessory.	With semi-skirted ABI consumable accessory.	Standard Skirt	Cirrus™Strips 0.1ml 8-tube strips		
	•		•	GeneAmp® 9800 FAST Block	Lifetech
	•		•	ProFlex™ 0.1ml (96-well block) FAST	
	•		•	Veriti 0.1ml (96-well block) FAST	
		•	•	T1 Thermocycler	BIOMETRA
			•	T3 Thermocycler	
		•	•	Tprofessional (Standard/Basic)+/- Gradient 96	
		•	•	TRobot, Uno	
			•	Uno II	
		•	•	Tadvanced Thermal cycler	
		•	•	C1000	BIO-RAD
			•	S1000	
		•	•	T100	
			•	Mini Gradient	BIO-RAD MJ RESEARCH
		•	•	PTC100™ (96-well block only)	
		•	•	DNA Engine™, DNA Dyad™, DNA Tetrad™	
		•	•	(Qiagen) Palm Cycler	Qiagen CORBETT
			•	MasterCycler®	Eppendorf
		•	•	MasterCycler® EP Gradient	
		•	•	MasterCycler® Gradient	
		•	•	Primus 96	MVG
		•	•	The Q Lifecycler	
		•	•	peqSTAR 96X universal / gradient / HPL	Peqlab
		•	•	labcycler	
		•	•	Robocycler 96 Gradient Cycler	STRATAGENE
		•	•	TP3000	sensoquest
		•	•	Cyclogene	TECHNE
		•	•	Flexigene	
		•	•	Genius, Genius Quad	
		•	•	TC412/512	
		•	•	Touchgene	
		•	•	Touchgene Gradient	
		•	•	MultiBlock System, MBS	THERMO Scientific (FISHER)
		•	•	PCR Sprint	
	•		•	7500 Fast, 7500 Fast Dx, 7900 Fast, 7900HT Fast	Lifetech
	•		•	StepOne™ and StepOnePlus™	
	•		•	QuantStudio™	
	•		•	ViiA7™	
		•	•	CFX96 Touch™	Bio-Rad
		•	•	Chromo4™	BIO-RAD MJ RESEARCH
		•	•	Opticon™, Opticon2™, MiniOpticon™	
		•	•	Mastercycler™ ep realplex	EPPENDORF
•			•	LightCycler® LC480	ROCHE
•			•	LightCycler® LC96	
			•	LightCycler® Nano	
			•	Quantica	TECHNE
		•	•	TOPTICAL Thermalcycler	BIOMETRA
		•	•	MegaBACE™ 1000 mark 2	AMERSHAM
		•	•	MegaBACE™ 500	