Cyxi™ PCR Master Mix
INSTRUCTIONS FOR USE

FLUOROGENICS LTD
Polymerase Chain Reaction Specialists

INTRODUCTION

Cyxi™ (Product code CYXI-1000-01) is a 'ready-to-go' pre-formulated master mix that may be used for a variety of Polymerase Chain Reaction (PCR) applications. **Cyxi™** has been formulated using a proprietary blend of sugars, stabilizers and macromolecules 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.

Cyxi™ contains the core reaction components required to carry out a PCR, this includes: core PCR reaction buffer, dNTP (inc dUTP) nucleotides, Magnesium ions and a high performance chemically-inactivated HotStart *Taq* DNA polymerase. Supplied as a dry reagent, **Cyxi™** is reconstituted using target-specific oligonucleotide primers and probes (not supplied) to provide a complete PCR reaction mix.

Highly flexible, the **Cyxi™** 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).

Cyxi™ may be reconstituted in a volume that allows more template to be added than conventional 2X frozen mixes. Each vial contains a 5% reagent excess and should be reconstituted to the following volumes:

Master Mix concentration req'd	Diluent Volume
10X	**105µL**
5X	**210µL**
2X	**525µL**
1X	**1050µL**

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

PROTOCOLS

Opening of **Cyxi™** Vial: Remove the foil cap and dispose of into a sharps container.

Gently tap the glass vial to settle contents that may have shifted during transit. Remove neoprene bung and add reagents as described in the following user protocols and vortex mix briefly.

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

The **Cyxi™** vial contents may be reconstituted to a final volume of 525µL to provide a 2X core reaction mix sufficient for 20x 50µL PCR reactions as follows:

Reagent	Volume
Cyxi™ vial *(Product code -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. 10-105µL 10µM stock solution e.g. 10-105µL 10µM stock solution
Oligonucleotide Probe *(not supplied)* Probe (TaqMan™/ Molecular Beacon/ Hybprobe etc) to a final reaction concentration 0.05 to 0.2µM	e.g. 25-105µL of 2µM stock
Diluent/ Nuclease-free water	To 525µL final Volume
TOTAL (for 2X reaction mix)	**525 µL**

The 2X reaction mix is then aliquotted in 25 µL volumes in PCR reaction tubes and supplemented with 25µL nucleic acid template ready for thermocycling. For smaller volumes substitute template with nuclease-free water to a final reaction volume of 50µL.

Thermal Cycling: The following are general recommended thermal cycler settings for **Cyxi™** 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)
Enzyme Activation	Hold	95	900	3-10
Amplification	Denature	95	5-10	3-10
	Anneal	50-65	5-30	3-10
	Extend	74	5-30	3-10
Strand Dissociation (Melt)	Hold	50	15	3-10
	Melt	95	10	0.1

Disposal: Dispose of according to local rules.

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Protocol 2: 20µL PCR reactions using 10µl template per reaction.

Reconstitute the **Cyxi™** reagent as described above using oligonucleotide primers/ probes and diluent to a 525µL final volume. Dispense 10µL of the reaction mastermix to individual PCR reaction tubes and supplement with 10µL template nucleic acid.

For smaller volumes of nucleic acid template, substitute with nuclease-free water to a final reaction volume of 20µL.

Protocol 3: Use of DNA binding dyes (SYBR®)

Cyxi™ reagent is compatible with most DNA intercalating dyes used for non-probe based real-time PCR applications. Following protocols 1 or 2, substitute the 0.05-0.2µM probe component for:

SYBR®Green/Gold* - 1:30,000 or greater final dilution of reference. *It may be necessary to also increase Magnesium concentration for optimum PCR performance due to free Magnesium abstraction by the minor-groove DNA binding dye.

ADDITIONAL INFORMATION

Cyxi™ reagent is formulated to provide 3mM final reaction Magnesium ions. This concentration can be adjusted to suit the requirements of different assays (typically in the range 3-5mM) by substituting diluent with additional $MgCl_2$ solution (not supplied) in the final master mix.

Cyxi™ 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. The addition of KCl to a final concentration of 10 mM may improve reaction performance for some applications.

STORAGE

Cyxi™ mastermix is supplied as a dried reagent and should be stored in its original packaging at 15-30°C.

Once reconstituted, the **Cyxi™** mastermix will remain stable for 24 hours if stored at 2-8°C. For longer term storage as a conventional mastermix reagent, **Cyxi™** mastermix should be 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 Hotstart Taq* polymerase (derived from *Thermus aquaticus*)
Nucleotides	dUTP proprietary mix
Buffer	Tris-HCl, pH 8.0
Magnesium Chloride	3 mM May be adjusted by user
Storage	15 to 30 °C
Shelf life	24 Months from manufacture date
Dissolution time	< 1s
Volume (final) upon dissolution	User defined >105µL. (Final reaction volume 1050µL)
BSA	Contains Bovine Serum Albumin of USA origin certified BSE free.

MANUFACTURER DETAILS

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