

<u>Distributor Information: Frequently Asked Questions (FAQs) for Cyxi™ and Generix™ products</u>

Storage and transport

1. How do I store Cyxi™ and Generix™?

Ambient temperature storage and transport for all Fluorogenics Ltd's products is standard without the use of a cold supply chain.

2. What about higher temperatures during transport?

Fluorogenics products can withstand transient excursions in temperatures up to 50°C during transport. Our recommended storage temperature for optimum product performance and longevity is 15-30°C.

3. Once dissolved how should I store the product I do not use?

Once dissolved the products are then normal X^* master mixes and may be stored short term in the fridge (2-8°C) for up to 24 hours, or in the freezer (-20°C) for up to 6 months. Up to 16%(v/v) glycerol (molecular grade) may be added to a 2X mixture to help reduce freeze thaw damage.

Resuspension

4. What do I resuspend the cake with, buffer or water?

Cyxi[™] and Generix[™] are provided with a complete formulation; only nuclease-free water is required to resuspend the mixture as the product is already buffered. Alternatively we can provide a suitable diluent to use (product code DIL-2000-01).

5. I have resuspened the cake in 1 mL but the PCR did not work?

All dry products supplied in glass lyovials are designed to provide 1 mL of $\underline{\text{final}}$ reaction $\underline{\text{mixture}}$ including all other components (primers and probes); e.g. 20 x 50 uL or 50 x 20 uL size. The reagents are provided dry to allow complete flexibility in how the user suspends the mixture. There is an excess of 5% reagent (see below).

6. Is there an excess in the lyovial?

All dry products supplied in glass lyovials are designed to be dissolved in a volume and provide a 5% excess such that the customer can withdraw enough reagent to have a complete 1 mL final reaction volume. For example, if the mix is resuspended to provide a 2X master mix then the volume of water to be added is 525 uL.

Enzymes

7. Why is Cyxi™FAST so Fast?

CyxiFAST range of products contain an *anti-Taq* antibody as a Hotstart and a rapid cycling formulation. Upon the first denaturing step of PCR, the Taq is completely activated. Other hot start enzymes (and products such as $Cyxi^{TM}$) use a chemically-inactivated polymerase. Although providing an excellent hotstart, the activation process is slow and requires up to 15 minutes initial hold at 95°C.



- 8. Does the *Taq* polymerase enzyme in the products have a 5 to 3 'exonuclease activity? Yes, all FGL products will perform the 5' nuclease assay (Taqman®). You may require end user rights to do so.
- 9. What is the principle of reverse transcriptase in Cyxi[™]FAST-RT? Cyxi[™]FAST-RT is a single tube solution for the generation of cDNA from RNA template and subsequent PCR amplification. It contains a blend of *MMuLV* and *Taq* Hotstart polymerase perfected to provide specific primer driven RT and PCR and a two–stage process (hold step at 48-50°C followed immediately by thermal cycling).
- 10. What enzyme performs the reverse transcriptase step in Cyxi™FAST-RT?

 This is a high performance thermostable *MMuLV*, an RNA dependant DNA polymerase. It can perform RT at much higher temperatures than native *MMuLV* allowing the RT step to be carried out at 48-50°C (rather than 42°C) for more efficient (specific) priming and reduce the effects of template secondary structure.

Dyes and Probes

- 11.Can Cyxi[™] and Generix[™] be used for normal (non real-time) PCR? Yes, these products may be used for most PCR applications.
- 12.Can Cyxi[™] and Generix[™] be used on capillary, including glass format PCR devices? Yes, these products are formulated for rapid PCR devices as well as standard plates. This includes glass formats such as the LightCycler® capillaries.
- 13.Can Cyxi[™] and Generix[™] be used with DNA binding dyes or probes?

 Yes. Both products are completely configurable for generic or probe based amplification. Cyxi[™]Green (product code GREEN-1500-01) is available from FGL for DNA binding detection. Other dyes such as SYBR®Green-1 may be added by the end-user. Fluorogenic probes such as Taqman®, Molecular Beacons, Scorpions® and others may be used providing you have end-user intellectual property rights to do so.
- 14. Does Cyxi™ and Generix™ contain a passive reference?

 No, the reason is the amount and type of the passive reference depend upon the real-time instrument to be used. However, FGL can supply an additive for this purpose for ABI and similar instruments.
- 15.Is Cyxi[™] and Generix[™] available with a DNA binding dye such as Sybr®Green-1?

 No, the reason is the amount and is dependent upon the instrument to be used. However, FGL can supply a supplement of Cyxi[™]Green that may be used product code GREEN-1500-01.

Multiplexing

16.Can Cyxi™ and Generix™ be used for multiplex PCR?

Yes, all products contain a formulation designed to optimise up to 4 targets in a2 single reaction. Greater multiplexing may be achieved through further solute additions. Please enquire.