10x GC-Rich PCR Enhancer

Technical Data Sheet

Catalogue	Pack Size
GCE-1	1 mL

For research and educational use only.

Description

DNA templates with a high proportion (>65%) of GC (guanine and cytosine) nucleotide base pairs can be difficult to amplify during PCR. This is because the strength of hydrogen bonding between these nucleobases is greater than that of the other nucleobases (adenine and thymine), and because GC-rich templates have a tendency to fold into complicated secondary structures (e.g. loops and hairpin structures). Such DNA templates can be resistant to melting in the denaturation steps during PCR, preventing accurate primer binding and polymerase amplification.

10x GC-Rich PCR Enhancer is used as a PCR additive for difficult to amplify GC-rich templates. The optimised solution modifies the melting behaviour of nucleic acids, and often enhances amplification of suboptimal PCR templates with high degrees of secondary structures and GC-rich regions.

Application Recommendations

PCR protocols

Use as an additive for PCR reactions with DNA templates of higher than 65% GC content, or for problematic DNA extracts that produce non-specific amplification that may be caused by high GC content.

Reagent Composition

10x GC-Rich PCR Enhancer.

Storage & Stability

Routine storage at –20ºC. Temporary storage at room temperature has no detrimental effects on the quality of this reagent. Optionally aliquot into sterile tubes and freeze to minimise potential microbial contamination after opening.

Shipping conditions

Shipped at room temperature.

Safety warnings and precautions

This product and its components are not considered hazardous in their given concentrations. However, as with all scientific reagents this product should be handled and stored with care as standard practice. Wear gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.

Quick Start Protocol

Use if working with known GC-rich DNA templates, or if non-specific amplification occurs. Optimise PCR reactions by testing a range of GC-Rich PCR Enhancer concentrations to determine the best concentration.

For a typical GC-Rich PCR Enhancer calibration of 20 µL PCRs using a 5x PCR Master Mix, we suggest:

1. Make a 1x Master Mix for 4 PCR reactions of 20 µL to accommodate final concentrations of GC-Rich PCR Enhancer ranging from 0 – 3x, using the volumes shown in the table below.

Reagent	Volume
5x Master Mix	20 µL
PCR grade water*	36 µL
Primer 1 (10 µM)	2 µL
Primer 2 (10 µM)	2 µL
DNA extract	10 µL
Total volume	**70 µL**

**PCR grade water can comprise nuclease-free water, autoclaved molecular grade water, and DEPC-treated water.*

2. Aliquot out 14 µL of 1x Master Mix into each of 4 PCR tubes labeled 0 – 3. The additional 14 µL is an excess in case of pipetting errors.

3. To increase the concentration of GC-Rich PCR Enhancer in each PCR you will need to successively add an extra 2 µL of the reagent to each reaction, making up the total added volume to 6 µL with PCR grade water. A suggested gradient is shown in the table below.

Final GC-Rich PCR Enhancer concentration	PCR grade water	10x GC-Rich PCR Enhancer
0 x	6 µL	0 µL
1 x	4 µL	2 µL
2 x	2 µL	4 µL
3 x	0 µL	6 µL

Suggestion: to save on pipette tip use you can pipette 20 µL of PCR grade water into a PCR tube; if this is used as a stock will allow you to reuse the same pipette tip for all samples. Pipette each droplet onto the inner side of PCR tubes to avoid touching the Master Mix and any back transfer of reagents into the working stock. Make a working stock of 20 µL of 10x

GC Enhancer and do the same. Then close each PCR tube and flick to mix, ensuring the reaction mix is at the bottom of the tube.

4. Run PCR and gel electrophoresis according to recommended settings, and compare the amplified DNA to determine the most appropriate GC-Rich PCR Enhancer concentration to use for this specific PCR application. This is usually the concentration that shows the clearest bands of the expected sizes with the least non-specific amplification.