For research purpose only. Not for use in diagnostic procedures for clinical purposes. For IN VITRO USE ONLY.

ISO 9001/14001 Certified Company

i-GCapture Solution [5x, for amplifying GC-rich template]

Cat. No. 25191 1 ml

DESCRIPTION

Templates with high-GC content are particularly difficult to amplify, due to their high melting temperatures, and may require additional measures beyond optimizing reaction conditions. Incomplete separation of DNA strands can adversely effect amplification efficiency. In addition, template secondary structure or un-melted GC-rich regions can prevent primer binding and enzymatic elongation.

Cosolvents (formamide, DMSO, and glycerol) that affect DNA melting temperature are used to amplify template with high-GC content, but reactions containing the cosolvents require significant optimization to prevent inhibition of *Taq* DNA polymerase.

i-GCapture Solution (5x) is the PCR cosolvent for amplification of sequences that are 50% to 90% GC without inhibiting *Taq* DNA polymerase.

STORAGE

Store at -20℃.

KIT CONTENTS

• *i*-GCapture Solution (5X)	1 ml

GENERAL REACTION MIXTURE for PCR (total 20 ㎕)

Template	1 ng-1 ㎍
Primer (F)	5-10 pmoles
Primer (R)	5-10 pmoles
i-Taq™ DNA Polymerase (5U/㎕)	0.2-0.5 ㎕
10x PCR Buffer	2 ㎕
i-GCapture Solution (5x)	4 ㎕
dNTP Mixture (2.5mM each)	2 ㎕
Sterilized distilled water	up to 20 ㎕

SUGGESTED CYCLING PARAMETERS

PCR cycle		Temp.	PCR product size		
			100-500bp	500-1000bp	1Kb-5Kb
Initial denaturation		94℃	2min	2min	2min
30-40 Cycles	Denaturation	94℃	20sec	20sec	20sec
	Annealing	50-65℃	10sec	10sec	20sec
	Extension	65-72℃	20-30sec	40-50sec	1min/Kb
Final extension		72 ℃	Optional. Normally, 2-5min		

Note : This "SUGGESTED CYCLING PARAMETERS" serves as a guideline for PCR amplification. Optimal reaction conditions such as PCR cycles, annealing temperature, extension temperature and incubation times, may vary and must be individually determined.

EXPERIMENTAL INFORMATION

• Effect on amplification of template with high-GC content

The *i*-GCapture Solution (5x) is a PCR additive for amplifying template with high-GC content. Therefore, the solution would be deleterious in amplification of template with low-GC content.

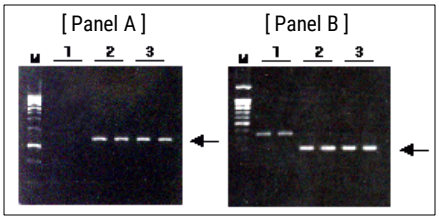

Figure 1. Effect of *i*-GCapture Solution (5x) on amplifying template with high-GC template using *i*-*Taq*™ DNA Polymerase.
[Panel A] 1.2kb region of the c-jun gene;
[Panel B] 180bp region of the retinoblastoma gene
PCR products were amplified from human genomic DNA in the absence (lane1) or presence (lane 2) of *i*-GCapture Solution (5x).
Lane M, 100bp & 1Kb Ladder DNA Marker; **lane 1,** In the absence of *i*-GCapture Solution (5x); **lane 2,** In the presence of *i*-GCapture Solution (5x); **lane 3,** PCR additive of company A.

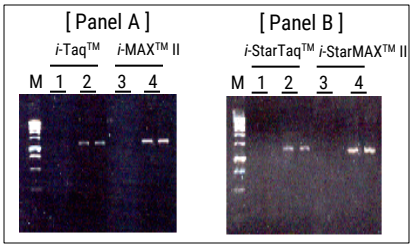

Figure 2. Comparison of effect on iNtRON's *Taq* Series.
[Panel A] Comparison of effect on *i*-Taq™ DNA Polymerase and *i*-MAX™ II DNA Polymerase.
[Panel B] Comparison of effect on *i*-StarTaq™ DNA Polymerase and *i*-StarMAX™ II DNA Polymerase.
1.2Kb region of c-jun gene was amplified from human genomic DNA in the absence (lane 1) or presence (lane 2) of *i*-GCapture Solution (5x) **Lane M,** 100bp & 1Kb Ladder DNA Marker; **lane 1, 3,** In the absence of *i*-GCapture Solution (5x); **lane 2, 4,** In the presence of *i*-GCapture Solution (5x)

Table 1. Primer Sequence

Target gene	Primer	Sequence (5'->3')
c-jun	Sense	GGGAGGGGACCGGGGAACAGAG
	Anti-sense	GAACAGTCCGTCACTTCACGTG
RB-1	Sense	CAGGACAGCGGCCCGGAG
	Anti-sense	CTGCAGACGCTCCGCCGT

Table 2. PCR profile

PCR cycle		Temp.	PCR product	
			c-jun (1.2Kb gene)	RB-1 (180bp gene)
Initial denaturation		94℃	2 min	2 min
30-40 Cycles	Denaturation	94℃	30 sec	20 sec
	Annealing	64℃		20 sec
		66℃	35 sec	
	Extension	72℃	40 sec	30 sec
Final extension		72 ℃	2 min	