VITA HIGH FIDELITY PCR KIT

FOR RESEARCH USE ONLY

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Vita High Fidelity PCR Kit provides a flexible and efficient solution for PCR applications that require an extra level of fidelity.
The unique blend of A-Family and B-Family polymerases combines favorable features from both worlds: the speed and high processivity of A-Family polymerases for fast and efficient amplification of target DNA (especially long and GC-rich targets) and the proofreading activity of B-Family polymerases for a decreased error rate. Together with the optimized 10X Reaction Buffer, *Vita High Fidelity PCR Kit* is suitable for a broad range of applications, such as PCR for sequencing, amplification of target sequences for cloning or site directed mutagenesis.

PRODUCT	SIZE	SKU
Vita High Fidelity PCR Kit	100 rxn / 50 µl	PCCSKU1024
	200 rxn / 50 µl	PCCSKU1025

STORAGE

Store *Vita High Fidelity PCR Kit* at -20°C upon arrival and avoid repeated freeze and thaw cycles. Stored under these conditions, reagents can be used until the expiry date stated on the packaging.

Vita High Fidelity PCR Kit comes with 150 / 300 µl PCR-grade dNTPs. Store at -20°C upon arrival and avoid repeated freeze and thaw cycles. Aliquot if necessary.

CONSIDERATIONS BEFORE STARTING

- Primers & template: Design primer pairs that exhibit similar melting temperatures to ensure specific amplification of your target. Primer sequences should furthermore be analyzed for hairpin or duplex formation as well as for secondary binding sites on the target. If possible, the GC-content of your primers should be 40%-60%.

- For high fidelity applications like site-directed mutagenesis, primers of a higher purity (e.g. HPLC purified) are recommended. Make sure to use high quality template DNA and adjust amount according to the type of template: use 1-10 ng per 25 µl reaction for low complexity, high copy targets (e.g.) plasmid DNA, use up to 100 ng per 25 µl reaction for high complexity, low copy targets (e.g. genomic DNA).

- Annealing: when working with new primers, the optimal annealing temperature must be determined empirically. Start with T_m – 20°C and lower the temperature stepwise until satisfactory amplification is achieved. If possible, run a gradient for the annealing step. Higher annealing temperatures increase specificity, lower annealing temperatures increase yield.

- For GC-rich or otherwise complex targets, up to 5% DMSO can be used as an additive to the PCR reaction.

A) PREPARING THE PCR

- Thaw all components completely and mix gently to ensure even distribution of components. Spin the tube quickly to collect all liquid at the bottom of the tube

- Prepare the reaction on ice and add primers last as the proofreading feature of *Vita High Fidelity PCR* Kit could lead to primer degradation.
- Transfer the PCR mix to nuclease-free tubes or plates, seal and spin quickly to collect all liquid at the bottom of the tube / well.

Component	Volume	Final Concentration
10X High Fidelity Buffer	5 µl	1X
dNTP Mix (10 mM each)	1 µl	0.2 mM each
Primer 1 (10 µM)	1 µl	0.1 µM – 0.5 µM
Primer 2 (10 µM)	1 µl	0.1 µM – 0.5 µM
High Fidelity Enzyme Mix	0.5 µl	
template DNA[1]	X µl	
dH_20	to 50 µl[2]	

[1] Use <1 ng for low complexity targets as a first approach, 10-100 ng for high complexity targets are recommended.
[2] Reaction volume can be reduced to 10 µl. Scale down reagents appropriately. Volumes >50 µl are not recommended.

B) Cycling protocol

Step	Cycles	Temperature	Duration
Initial Denaturation	1	94°C	5 minutes
Amplification	15-35[1]	94°C	30 seconds
		T_m – 5°C	30 seconds
		72°C	30 seconds / kb[2]
Final Extension	1	72°C	5 minutes

[1] The highest fidelity can be achieved with ≤25 cycles. If the yield is low due to low efficiency or template concentrations, 30-35 cycles produce enough material for downstream applications.
[2] Extension times of 1 min/kb may be necessary for high complexity targets.

C) Analysis

Analyze the amplification reaction by gel electrophoresis using an acrylamide or agarose gel of appropriate percentage or directly process the reaction for downstream applications.

D) Troubleshooting

Result	Possible Explanation	Solution
No amplification / poor amplification	Cycling protocol	Increase the extension time up to 1 min/ kb
		Increase the number of cycles
		Reduce the annealing temperature or run a gradient
	dNTPs	dNTPs degrade when exposed to frequent freeze and thaw cycles. Use the supplied dNTPs and make aliquots if necessary
	Template quality & quantity	Excess DNA can chelate Mg^{2+}. Perform a template dilution to determine the optimal concentration.
Non-specific amplification / smearing	Cycling protocol	Avoid unnecessary long annealing and extension times as they can provoke non-specific primer binding.
		Increase annealing temperature stepwise

	Primer concentration	Decrease primer concentration
	Enzyme	0.5 µl Enzyme Mix for 50 µl reaction is sufficient for most applications. Excess enzyme can cause band smearing.
	GC-content	When working with a GC rich target, addition of 5% DMSO can improve amplification.