



VITA HIGH FIDELITY KIT & MASTERMIX

FOR RESEARCH USE ONLY

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Vita High Fidelity Kit provides a flexible and efficient solution for PCR applications that require an extra level of fidelity. For standardized applications, Vita High Fidelity Kit is available as a convenient 2X mastermix formulation.

Vita High Fidelity Kit comes with 150 / 300 µl PCR-grade dNTPs (10 mM each).

PRODUCT	SIZE	SKU
Vita High Fidelity Kit	100 rxn / 50 µl	PCCSKU1024
	200 rxn / 50 µl	PCCSKU1025
Vita High Fidelity 2X Mastermix	1 ml 2X Mastermix	PCCSKU1026
	5 ml 2X Mastermix	PCCSKU1027

STORAGE

Store at -20°C upon arrival and avoid repeated freeze / thaw cycles. Stored under these conditions, reagents can be used until the expiry date stated on the packaging. Make aliquots if necessary.

A) PREPARING THE PCR

- Thaw all components completely and mix gently.
- Prepare the reaction on ice and add primers last as the polymerase's proofreading activity can lead to primer degradation.
- Transfer the PCR mix to nuclease-free tubes or plates, seal and spin down quickly

COMPONENT	VOLUME	FINAL CONCENTRATION
10X High Fidelity Buffer / Vita High Fidelity 2X Mastermix	5 µl / 25 µl	1X
dNTP Mix (10 mM each)	1 µl / ----	0.2 mM each
Primer 1 (10 µM)	1 µl / 1 µl	0.1 µM – 0.5 µM
Primer 2 (10 µM)	1 µl / 1 µl	0.1 µM – 0.5 µM
High Fidelity Enzyme Mix	0.5 µl / ----	
template DNA ¹	X µl	
dH ₂ O	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.