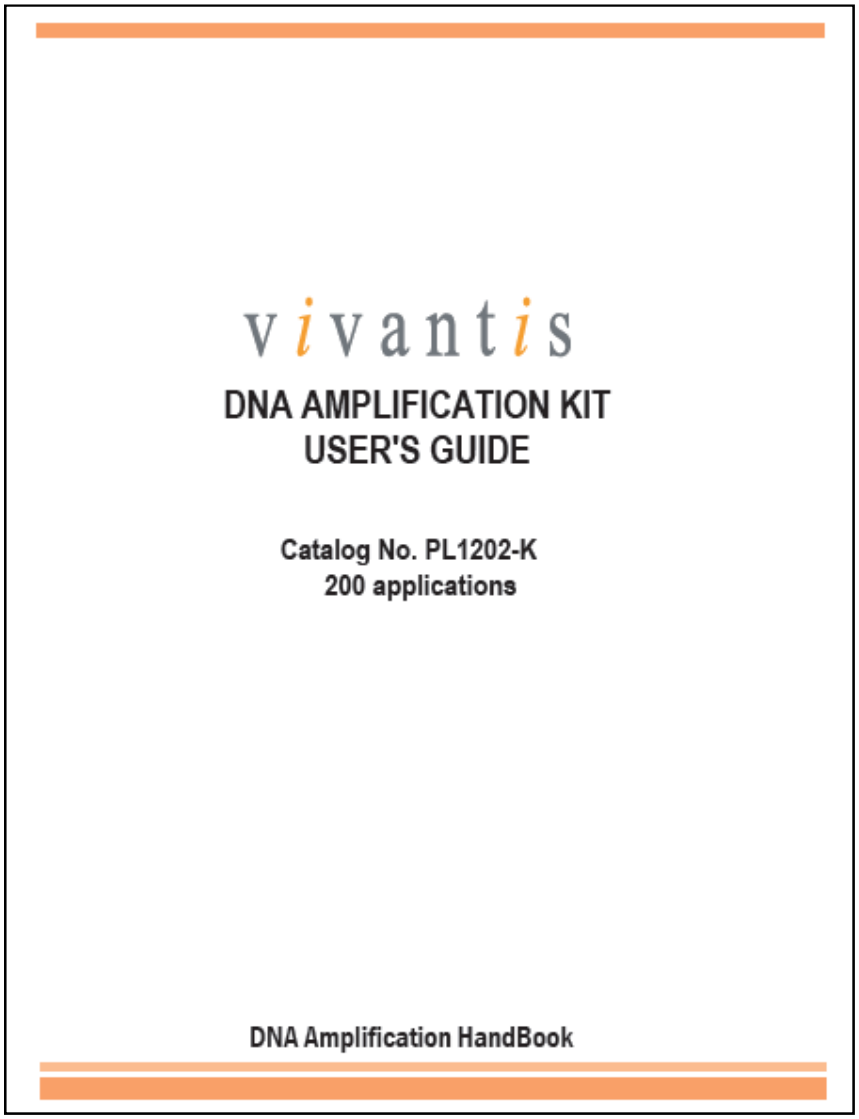

INTRODUCTION

The DNA Amplification Kit is designed to contain high quality *Taq* DNA Polymerases, buffers, dNTPs mix, and nuclease-free water as a complete set for user's convenience in performing DNA amplification experiments. The kit allows for up to 200 applications or more in a 50µl PCR reaction. DNA and primers are also provided as a positive control for users to carry out PCR using the recommended parameters as shown in this manual.

The kit is also supplied with DNA ladders (ready-to-use) for up to 100 applications to determine the size of PCR products or other double-stranded DNA fragments during gel electrophoresis.

KIT COMPONENTS

PCR Amplification Reagents

Taq DNA Polymerase (5u/µl)	500u
10X ViBuffer A (500mM KCl, 100mM Tris-HCl (pH 9.1) and 0.1% Triton X-100)	2ml
10X ViBuffer S (160Mm $(NH_4)_2SO_4$, 500mM Tris-HCl (pH9.2), 17.5mM $MgCl_2$ and 0.1% Triton X-100)	1ml
50mM $MgCl_2$	1ml
2mM dNTPs Mix	1ml
Control DNA (5ng/µl)	100ng
10µM Forward primer	25µl
10µM Reverse primer	25µl
Nuclease-free water	1ml X 2

DNA Ladder (ready-to-use)

VC 100bp plus (0.1µg/µl)	100 applications
VC 1kb (0.1µg/µl)	100 applications
6X loading dye	1ml

STORAGE & STABILITY

Store all components at -20°C

Kit components are guaranteed to be stable for 2 year from the date of manufacture.

PCR PROTOCOL (Control DNA)

1. Gently mix all solutions after thawing. Keep solutions on ice from this point onwards.
2. Add the following reagents into a PCR tube, on ice.

Reagent	Quantity (µl)	Final Concentration
Water, nuclease-free	38.1	-
10X ViBuffer A	5.0	1X
2mM dNTPs mix	2.0	0.08mM
50mM $MgCl_2$	1.5	1.5mM
10uM Forward Primer	1.0	0.2µM
10uM Reverse Primer	1.0	0.2µM
Control DNA (5ng/µl)	1.0	5ng
Taq DNA Polymerase (5u/µl)	0.4	2unit
Total Volume	50.0	

3. Gently mix the PCR reagents. Briefly centrifuge the tubes to collect the contents at the bottom of the tube.
4. Perform DNA amplification using the following program:

Segment	No. of cycles	Temperature	Duration
1	1	95°C	3 min
2	30	95°C	30 sec
		52°C	30 sec
		72°C	30 sec
3	1	72°C	5 min
	1	4°C	pause

5. Run 5µl of the PCR products along with 0.3 – 0.5µg of VC100bp plus DNA ladder in a 1.0% agarose gel. Stain gel with EtBr to visualize DNA bands under UV.

Note: The PCR product of the positive control should provide a 1.4kb DNA fragment size.

TROUBLESHOOTING

PCR Reactions

Problems	Possibility	Suggestions
Low yield or no PCR product	Missing component in reaction	Check the reaction components and repeat the reaction.
Multiple, non-specific amplification products	Cross contamination of DNA	Use a separate workplace and pipettes for PCR. Wear gloves at all times.
	Excessive amounts of enzyme used	Decrease amount of *Taq* DNA Polymerase in the reaction tube.
	Excessive amounts of DNA template used	Decrease amount of DNA template in the reaction tube.
	Excessive number of cycles	Reduce number of cycles.
	Excessive amount of $MgCl_2$ used	Decrease the concentration of $MgCl_2$ in the reaction tube.
	Long extension time	Reduce extension time.
	Pipetting error	Perform PCR in reaction master mixes.
Smearing of PCR product when viewed after gel electrophoresis	Agarose gel used was not fresh	Repeat electrophoresis with fresh agarose gel.
	Insufficient amount of $MgCl_2$ used	Increase the concentration of $MgCl_2$ in the reaction tube.
	Excessive amounts of enzyme used	Decrease amount of *Taq* DNA Polymerase in the reaction tube.