

PCR

KOD Neo Plus

To prepare several parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by mixing water, buffer, dNTPs, primers and template DNA. KOD Neo Plus DNA polymerase should be the last component added. Prepare sufficient master mix for the number of reactions plus one extra to allow for pipetting error.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 50 μ l reaction:
variable
3. Gently vortex the samples and spin down.
4. If using a thermal cycler that does not use a heated lid, overlay the reaction mixture with 25 μ l of mineral oil.
5. Perform PCR using the following thermal cycling conditions

10X Buffer	5 μ l
MgCl ₂	3 μ l
2mM dNTP	5 μ l
Primer 1	1.5 μ l
Primer 2	1.5 μ l
DNA template	< 1 μ g
KOD Neo Puls(1U/ μ l)	1 μ l
Water, nuclease-free	up to 50 μ l

	Temperature/ $^{\circ}$ C	Time	Number of cycles
Initial denaturation	94	2 min	
Denaturation	98	10s	25-45 cycles
Annealing	T _m	30s	
Extension	68	30s/kb	
Final extension	68	3min	