

2 x Es Taq Master Mix

E665629

Storage at -20°C

Introduction:

Es Taq Master Mix is a 2X concentrated solution of Es Taq DNA Polymerase, Mg^{2+} , dNTPs and all other components required for PCR, except DNA template and primers. This pre-mixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up. The mix is optimized for efficient and reproducible PCR. Its application for routine PCR with high reproducibility and generation of PCR products for TA.

Ordering Information:

Cat No.	Component	E665629-5mL	E665629-25mL	Storage
E665629A	2 × Es Taq MasterMix	5×1mL	5×5mL	-20°C. Avoid freeze/thaw cycle
E665629B	dd H ₂ O	5×1mL	5×5mL	-20°C.

Notes: 2 × Es Taq MasterMix contains Es Taq DNA Polymerase, 3 mM $MgCl_2$ and 400 μ M each dNTP

Protocol:

Gently vortex and briefly centrifuge 2 × Es Taq MasterMix after thawing. Place a thin-walled PCR tube on ice and add the following components for each 50 μ L reaction:

PCR reaction:

Components	Total volume:50 μ L	Concentration
2×Es Taq Master Mix	25 μ L	1 ×
Forward Primer, 10 μ M	2 μ L	0.4 μ M
Reverse Primer, 10 μ M	2 μ L	0.4 μ M
Template DNA	<0.5 μ g	<0.5 μ g/50 μ L
ddH ₂ O	up to 50 μ L	

Notes: The recommended concentration range of the PCR primers is 0.1-1 μ M. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products.

PCR thermal cycling conditions :

Step	Temperature	Time	Number of cycles
Initial denaturation	94° C	2 min	
Denaturation	94° C	30 s	25-35
Annealing	55-65°C	30 s	
Extension	72° C	30 s	
Final Extension	72° C	2 min	

Notes :

- 1) The annealing temperature should be 5°C lower than the melting temperature (T_m) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments.
- 2) The optimal extension temperature for ES Taq DNA polymerase is 70-75°C. The recommended extension step is 2 min/kb at 72°C for PCR.
- 3) If less than 10 copies of the template are present in the reaction, about 40 cycles are required. For higher template amounts, 25-35 cycles are sufficient.