DATA SHEET

Jena Bioscience

Crystal Hot Start Master Lyophilisate

lyophilised Hot Start Master Mix

Cat. No.	Amount
PCR-153S	192 reactions x 20 µl
PCR-153L	960 reactions x 20 µl

For *in vitro* use only!

Shipping: shipped at ambient temperature

Storage Conditions: store at ambient temperature

Additional Storage Conditions: Store in an aluminium-coated bag or on a dry place.

Lyophilisates may hydrate at humidity levels >70 % when sealing is opened.

Shelf Life: 12 months

Description:
Hot Start Master Lyophilisate is delivered in PCR reaction tube strips or 96-well plates preloaded with a complete hot start master mix in a dry, room temperature stable format. The lyophilisate combines highest performance with convenience of use and stability. There is no need for freezing, thawing or pipetting on ice. The few remaining pipetting steps minimize the risk of errors or contaminations.
Each vial contains polymerase, dNTPs and reaction buffer with $MgCl_2$ required for a 20 µl PCR assay.
To perform PCR, fill up the vials with a premix of primers and PCR-grade water and add DNA template. If necessary, centrifuge to remove bubbles, vortex the vials to assure homogeneity and start cycling.
The Hot Start Master Lyophilisate provides improved specificity and sensitivity when amplifying low-copy-number targets in complex backgrounds. The polymerase activity is blocked at ambient temperature and switched on automatically at the onset of the initial denaturation. The thermal activation prevents the extension of nonspecifically annealed primers and primer-dimers formed during PCR setup.

Content:
Hot Start Master Lyophilisate
Preloaded lyophilisates of hot start DNA polymerase, dATP, dCTP, dGTP, dTTP, Reaction Buffer with $MgCl_2$ and stabilizers.

PCR grade water

Activation step
Hot Start Master Lyophilisate requires no prolonged heating or denaturing step. The polymerase inhibiting ligand is quickly released at the increased temperature of thermal cycling.

Recommended 20 µl PCR assay:

forward Primer	0.2 - 1 µM (0.4 - 2 µl / 10 µM)
reverse Primer	0.2 - 1 µM (0.4 - 2 µl / 10 µM)
template DNA	1 - 50 ng
PCR-grade water	fill up to 20 µl

Recommended cycling conditions:

Initial denaturation	94 °C	2 min	1x
Denaturation	94 °C	30 sec	30x
Annealing[1)]	45 - 68 °C	30 sec	30x
Elongation[2)]	72 °C	30 sec - 3 min	30x
Final elongation	72 °C	2 min	1x

[1)] The annealing temperature depends on the melting temperature of the primers used.

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[2)] The elongation time depends on the length of the fragments to be amplified. A time of 1 min/kb is recommended.

For optimal specificity and amplification an individual optimization of the recommended parameters may be necessary for each new primer-template pair.