2x PCR Hypernova-RED

PCR Master Mix

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2x PCR Hypernova-RED is a 2x concentrated, ready-to-use PCR master mix which facilitates an easy and rapid PCR reaction set up. The ***2x PCR Hypernova-RED*** solution contains a reaction buffer, magnesium chloride, dNTPs, ***Hypernova*** polymerase isolated from *Pyrococcus woesei* and enzymes increasing amplification efficiency. In order to set up the PCR reaction, all that needs adding to the master mix is the template, the primer set and water. This convenient ***2x PCR Hypernova-RED*** master mix reduces the time required to set up PCR reactions and decreases the possibility of contamination, particularly when preparing large numbers of reactions. Moreover, the master mix is supplemented with an inner dye and a density reagent, which allows for direct loading of PCR products to a gel.

Features and advantages

- → Increased processivity (for long amplicons, up to 10 kb)
- → High fidelity (proofreading activity)
- → Ideal for difficult templates which fail with standard *Taq* DNA polymerases
- → Fool proof during multiplex PCR
- → Very specific and sensitive
- → More thermostable than *Taq* polymerase
- → Facilitate high throughput PCR reaction set-up
- → Reduced risk of contamination
- → Direct gel loading

Applications

- → Reproducible amplification of long templates
- → Multiplex PCR
- → Cloning, site-directed mutagenesis and other methods which require high fidelity
- → Amplification of regions abundant in GC

Protocol

1. Prior to use, thaw the Master Mix solution and other reagents completely, mix thoroughly and spin briefly.
2. Prepare reaction mixtures according to the following suggested reaction set-up:

Reagent	Suggested amount per reaction	Acceptable final concentrations in reaction mixture
2x PCR Hypernova-RED	**25 µl**	**1x**
10 µM Forward primer	1 µl	0.1 – 1 µM
10 µM Reverse primer	1 µl	0.1 – 1 µM
DNA template	1 – 100 ng	10 pg – 0.5 µg
PCR-grade water	fill up to 50 µl	fill up to required volume

This composition is intended for use as a guide only; conditions may vary from reaction to reaction and may require optimization.

3. Mix the prepared reaction mixture thoroughly by pipetting or vortexing, then spin briefly.
4. Place the prepared PCR mixture in a thermal cycler and start the PCR reaction.
5. After reaction has finished, apply reaction mixtures directly onto a gel.

Troubleshooting

For problems which may be encountered during PCR reaction set up and analysis, possible causes and solutions see: www.blirt.eu.

2x PCR Hypernova-RED
PCR Master Mix

Components	RP85 100 reactions (50 µl)	RP85-10 1000 reactions (50 µl)
2x PCR Hypernova-RED PCR Master Mix	2 x 1.25 ml	20 x 1.25 ml
PCR – grade water	2 x 1.5 ml	20 x 1.5 ml

Formulation

2x concentrated PCR reaction buffer, 4 mM $MgCl_2$; 1.6 mM dNTPs mix (0.4 mM of each dNTP); 0.08 U/µl *Hypernova* DNA Polymerase, red inner dye and density reagent.

Quality control

Free of unspecific nucleases (DNases). Extensively tested for PCR applications.

Storage & shipping

Storage conditions
Store all components at -20°C.

Shipping conditions
Shipping on dry or blue ice.

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