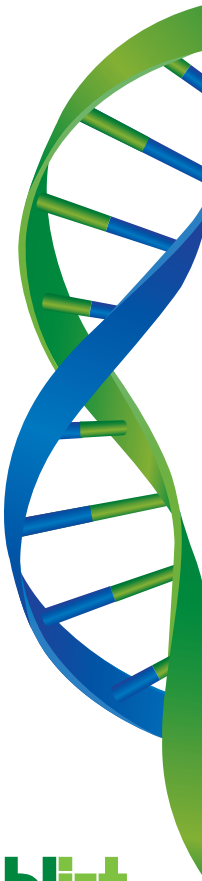
Hypernova

DNA Polymerase



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DNA Polymerase

The ***Hypernova*** DNA Polymerase is a recombinant, thermostable and proofreading *Pwo* DNA polymerase, originally isolated from the hyperthermophilic archaeon *Pyrococcus woesei*. The enzyme can generate very long amplicons (up to 10 kbp). ***Hypernova*** is a versatile and easy-to-use polymerase, since it works with many different protocols and requires minimal time consuming optimization.

The ***Hypernova*** polymerase catalyses a DNA replication reaction at 72°C. The halftime of the polymerase at 95°C is over 8 hours. It has 3′→ 5′ exonuclease activity (proofreading activity). No 5′→ 3′ exonuclease activity increases stability of the PCR products. It leaves blunt-ended 3′ endings (important in molecular cloning).

The polymerase is recommended for the multiplex PCR as it works well in a wide range of Mg^{2+}, salt concentration and pH. It is also recommended for the amplification of difficult templates (regions abundant in GC, palindromes and multiple repeats).

Features and advantages

- Increased processivity (for long amplicons, up to 10 kbp)
- High yield with minimal amounts of enzyme and little optimization
- 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

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

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Protocol

1. Prior to use, thaw the reagents completely, mix thoroughly and spin briefly.
2. Add the following reaction reagents to a sterile nuclease-free PCR Eppendorf tube:

Reagent	Suggested amount per reaction	Acceptable final concentrations in reaction mixture
10x *Hypernova*	5 µl	1x
8 mM dNTPs Mix	5 µl	0.2–0.25 mM of each dNTP
50 mM $MgCl_2$	2 µl	2–5 mM
10 µM Forward primer	1 µl	0.1–1.0 µM
10 µM Reverse primer	1 µl	0.1–1.0 µM
DNA template	1–100 ng	10 pg–0.5 µg
***Hypernova* DNA Polymerase**	**1 U**	**1–2 U**
PCR-grade water	fill up to 50 µl	

This composition is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimizing.

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. The table below shows a sample PCR cycling conditions.

Step	Temperature	Time	
Initial denaturation	95°C	1–5 min [1]	
Denaturation	95°C	30 s	30–40 cycles [4]
Annealing	45–65°C [2]	30 s	
Extension	72°C	30 s–10 min [3]	
Final extension	72°C	1–5 min	
Cooling	4°C	∞	

1) The initial denaturation time depends on the GC content within the amplified region and the template DNA type. For non-complex templates, such as plasmid DNA or cDNA, the initial denaturation step, carried out briefly (1–2 min), is recommended. For more complex templates, such as eukaryotic genomic DNA, a longer initial denaturation step (3–5 min) is required.
2) The annealing temperature depends on the primer sequences and their melting temperature (Tm). The optimal annealing temperature is usually 2–5°C below the Tm of primers.
3) The elongation time depends on the length of an amplified DNA fragment. Setting 60 seconds per 1 kbp of the PCR product is recommended.
4) The number of cycles depends on the number of copies of the amplified gene fragment. Thirty cycles is sufficient for low complexity templates. In the case of high complexity templates or less concentrated DNA, the number of cycles should be increased to forty.

Additional information

Both reaction buffers provided may be used with ***Hypernova*** DNA polymerase. **10x *Hypernova*** buffer is recommended as first approach and for applications requiring high specificity. **10x *Shark*** buffer is recommended for applications where high sensitivity and amplification efficiency is required (e.g. for amplification of multiple products). Both buffers may be evaluated to determine the buffer most suitable for specific application.

Troubleshooting

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

Storage buffer

20 mM Tris-HCl (pH 7.4, 25°C), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 50% (v/v) glycerol

Reaction buffers

10 x *Hypernova*
100 mM Tris-HCl (pH 8.3, 25°C), 500 mM KCl, 1.5% Triton X-100

10 x *Shark*
200 mM Tris-HCl (pH 8.3, 25°C), 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 1.0% Triton X-100

Unit definition

One unit is defined as an amount of enzyme required to incorporate 10 nmol of dNTPs to an insoluble DNA fraction in 30 minutes at 72°C in a 50 µl reaction.

Quality control

Extensively tested in various PCR reactions. Free of unspecific nucleases.

Hypernova DNA Polymerase

Components	RP232 200 U	RP235 1000 U	RP232-S 20 U
Hypernova **2 U/µl** DNA Polymerase	100 µl	500 µl	10 µl
10x *Hypernova* Reaction Buffer	1.25 ml	4 x 1.25 ml	100 µl
10x *Shark* Reaction Buffer	1.25 ml	4 x 1.25 ml	100 µl
50 mM $MgCl_2$	1 ml	4 x 1 ml	80 µl

Storage & shipping

Storage conditions
Store all components at -20°C.

Shipping conditions
Shipping on dry or blue ice.

For research use only

Expiry
Information on the label

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