PCR Reagents

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TaqNova Polymerase Family:

- → Validated for a wide range of templates and applications
- → Extreme yield even with minimal amounts of enzyme and low DNA concentrations
- → Available also for direct gel loading of PCR products, for specific amplification of difficult templates (hot-start) and as ready-to-use all-in one tube master mixes

PCR REAGENTS

We provide a wide range of reagents used in various PCR techniques, including:

- Thermostable DNA polymerases along with reaction buffers and magnesium salts for reaction optimisation:
  - ***TaqNova*** DNA Polymerase (*Taq*)
  - ***TaqNovaHS*** DNA Polymerase (hot-start *Taq*)
  - ***TaqNovaGC*** DNA Polymerase (GC-rich templates)
  - ***Hypernova*** DNA Polymerase (*Pwo*, proofreading)
  - ***LongNova*** DNA Polymerase (long range PCR)

- **dNTPs** available as an equimolar mixture of **dATP**, **dCTP**, **dGTP** and **dTTP** or as a set of four separate single-nucleotide solutions

- various PCR enhancers:
  - ***PCR Anti-inhibitor***
  - ***TaqSSB***, ***TneSSB***, ***TmaSSB proteins***
  - ***5x GC-Additive***

- ready-to-use PCR master mixes containing all the necessary PCR reagents in a single tube:
  - ***2xPCR TaqNova-RED***
  - ***2xPCR Hyprnova-RED***
  - ***2xPCR LongNova-RED***

We also offer design and/or synthesis of PCR primers and molecular probes for the Real-Time PCR.

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It is very important for the user to choose the polymerase most suited to their application. A polymerase selection table is provided to facilitate your choice.

	PRODUCT	PROPERTIES						APPLICATIONS									
		Template length	Hot-start	Proofreading activity	High processivity	Mixes	Direct gel loading	Routine PCR	High specificity assays	High-fidelity PCR	Difficult templates	GC-rich templates	Diagnostic PCR	Long Range PCR (> 10 kb)	Site-directed mutagenesis	Blunt-end cloning	TA cloning
DNA POLYMERASES	*TaqNova*	< 5 kb				✓		✓					✓				✓
DNA POLYMERASES	*TaqNova-RED*	< 5 kb				✓	✓	✓					✓				✓
DNA POLYMERASES	*TaqNovaHS*	< 5 kb	✓						✓		✓		✓				✓
DNA POLYMERASES	*TaqNovaGC*	< 5 kb									✓	✓	✓				✓
DNA POLYMERASES	*Hypernova*	< 10 kb		✓	✓	✓				✓	✓		✓		✓	✓	
DNA POLYMERASES	*Hypernova-RED*	< 10 kb		✓	✓	✓	✓			✓	✓		✓		✓	✓	
DNA POLYMERASES	*LongNova*	< 20 kb		✓	✓	✓				✓				✓	✓		✓
DNA POLYMERASES	*LongNova-RED*	< 20 kb		✓	✓	✓	✓			✓				✓	✓		✓
ENHANCERS	*PCR Anti-inhibitor*										✓						
ENHANCERS	*SSB Proteins*								✓		✓	✓					
ENHANCERS	*5x GC-Additive*										✓	✓					

TaqNova Polymerase

TaqNova DNA Polymerase is a 94 kDa recombinant, thermostable *Taq* DNA polymerase isolated from *Thermus aquaticus*. It is recommended for a wide range of applications, which require DNA synthesis in extremely high temperatures. The *TaqNova* polymerase is a universal and easy-to-use DNA polymerase, which works rapidly and effectively in various PCR conditions. The enzyme catalyses DNA synthesis in a 5'→3' directions, it does not show a 3'→5' exonuclease activity, however it has a 5'→3' exonuclease activity.

- → Consistent results
- → Suitable for a wide range of applications
- → Extreme yield with minimal amounts of enzyme and little optimisation
- → Half-life of the enzyme is 45 minutes at 95°C
- → Amplifies fragments of up to 5 kb
- → Leaves ´A´ overhangs

Applications:

- → Fast and efficient amplification of short and medium size DNA sequences
- → Diagnostic PCR
- → High-troughput PCR

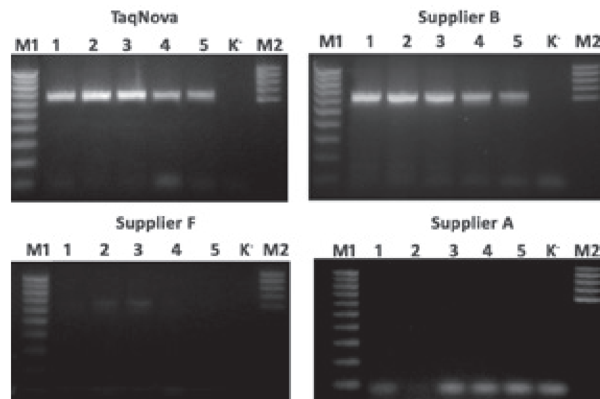


Fig. 1 ***Efficiency and sensitivity of Taq polymerases.***
A 630 bp fragment of *ccr5* gene was amplified using the *TaqNova* polymerase and results were compared with the results obtained in a parallel reaction using *Taq* polymerases from supplier B, supplier F and supplier A. The process used serial dilutions of human genomic DNA, incubated for 2 min at 95°C, followed by 40 cycles of 30 sec at 95°C, 30 sec at 54°C, 30 sec at 72°C and a final extension 2 min at 72°C. The DNA ladders used were M100-1000 and M600-1000. *TaqNova* delivers higher or comparable yields and is more or equally sensitive comparing to 3 competing products.

Quick and simple PCR set-up

We propose ***TaqNova-RED*** DNA Polymerase and ***2x PCR TaqNova-RED*** to simplify PCR operations. This is vital, especially for routine applications e.g. diagnostic assays.

TaqNova-RED DNA Polymerase

The *TaqNova-RED* DNA Polymerase consists of *TaqNova* DNA polymerase with an inert red dye to facilitate accurate low volume pipetting and as an indicator of an enzyme addition. This dye has no adverse effect on the outcome of PCR; yields are the same as with standard *TaqNova* DNA polymerase. Use of the *TaqNova-RED* DNA Polymerase decreases the risk of making a mistake during a reaction set-up e.g. skipping the polymerase, inaccurate reagents mixing. Additionally a PCR product can be applied directly onto a gel after amplification without mixing with loading buffer.

- → **Facilitate PCR reaction set-up**
- → **Reduced risk of mistake, thanks to the inert red dye**
- → **Direct gel loading**

2x PCR TaqNova-RED – PCR Mix

The *2x PCR TaqNova-RED* contains *TaqNova* DNA polymerase, dNTPs, optimal PCR buffer and all other components required for PCR, except for DNA template and primers. The *2x PCR TaqNova-RED* application reduces the number of pipetting steps and contamination risk, facilitates greater efficiency, throughput and reproducibility. The PCR mixtures prepared with the *2x PCR TaqNova-RED* can be applied onto a gel directly after amplification, no loading buffer use needed. It saves time and facilitates agarose or polyacrylamide gel electrophoresis preparation.

- → **Facilitate PCR reaction set-up**
- → **Reduced risk of contamination**
- → **Direct gel loading**

Hot-start PCR

TaqNovaHS DNA Polymerase is a mixture of thermostable *TaqNova* DNA polymerase isolated from *Thermus aquaticus* and a highly specific monoclonal antibody, which acts as an inhibitor of the polymerization activity. The *TaqNovaHS* enables easy set up of a hot-start PCR reaction at room temperature. The antibody binds reversibly to the enzyme, inhibiting polymerase activity at ambient temperatures, which prevents extension of nonspecifically annealed primers and primer dimers formed at low temperatures during PCR setup. The antibody is released from the polymerase during normal cycling conditions. The use of the *TaqNovaHS* DNA Polymerase does not require any additional incubation step to activate the enzyme. It is recommended for a wide range of demending applications which require highly specific amplification. The *TaqNovaHS* DNA polymerase is a universal and easy-to-use DNA polymerase which works rapidly and effectively in various PCR conditions. The enzyme catalyses DNA synthesis in a 5'→3' direction, shows no 3'→5' exonuclease activity, but has a 5'→3' exonuclease activity.

Features:

- High PCR specificity with minimal optimisation
- Minimises amplification of non-specific products and primer-dimers
- Fast 3-minute enzyme activation time
- Suitable for a wide range of applications
- Increased yield of PCR products
- Amplifies fragments of up to 5 kb
- Leaves ´A´ overhangs

Applications:

- Hot-start PCR
- Singleplex and multiplex PCR
- Diagnostic PCR with DNA from various kinds of specimen
- Specific amplification of difficult templates (GC-rich)

Ordering information

PRODUCT	CONCENTRATION	VOLUME	CAT. NO.
TaqNova DNA Polymerase	2 U/µl	200 U	RP702
		500 U	RP705
		1000 U	RP710
		2500 U	RP725
	5 U/µl	200 U	RP702A
		500 U	RP705A
		1000 U	RP710A
		2500 U	RP725A
TaqNova-RED DNA Polymerase	1 U/µl	200 U	RP20R
		1000 U	RP100R
2x PCR TaqNova-RED	0.04 U/µl	100 reactions	RP85T
		1000 reactions	RP85T-10
TaqNovaHS Hot-start DNA Polymerase	2 U/µl	200 U	RP902
		500 U	RP905
		1000 U	RP910
		2500 U	RP925
	5 U/µl	200 U	RP902A
		500 U	RP905A
		1000 U	RP910A
		2500 U	RP925A
TaqNovaGC DNA Polymerase	5 U/µl	200 U	RP73-020
		1000 U	RP73-100

GC-rich templates

Successful amplification depends of GC content and the complexity of the DNA template. The ***TaqNovaGC*** DNA Polymerase is an ideal tool for amplification of GC-rich templates. Special Buffer – ***5x GC-Additive*** changes DNA behaviour upon heating and can be used with primer – template pairs with GC-rich content which do not work well with standard PCR conditions.

The *5x GC-Additive* reduces the number of secondary structures and enables specific hybridization of primers.

- **GC-rich templates**
- **High specificity**
- **Ideal for problematic templates, which fail with standard *Taq* DNA polymerases**

Hypernova Polymerase

Hypernova DNA Polymerase is a unique blend of a highly thermostable and proofreading modified DNA polymerase *Pwo* isolated from the hyperthermophilic archaeon *Pyrococcus woesei* and enzymes increasing yield and performance of the PCR reaction. The enzyme mix can generate very long amplicons (up to 10 kb). *Hypernova* is a versatile and easy-to-use polymerase, since it works with many different protocols and requires minimal time consuming optimisation. The polymerase produces higher yields than most commercially available enzymes and is ideally suited for difficult PCR templates. The polymerase maintains 95% activity even after 40 cycles consisting of three one minute steps each.

Features:

→ High processivity (for long amplicons)
→ High yield with minimal amounts of enzyme and little optimisation
→ High fidelity (proofreading activity)
→ Mistake-proof during multiplex PCR
→ Very specific and sensitive
→ More thermostable than *Taq* polymerase

Ordering information			
PRODUCT	CONCENTRATION	VOLUME	CAT. NO.
Hypernova DNA Polymerase	2 U/µl	200 U	RP232
		1000 U	RP235
Hypernova-RED DNA Polymerase	1 U/µl	200 U	RP232R
		1000 U	RP235R
2x PCR Hypernova-RED	0.04 U/µl	100 reactions	RP85
		1000 reactions	RP85-10

Applications:

→ Long range PCR
→ High fidelity for purposes of blunt-end PCR cloning, site-directed mutagenesis, etc.
→ Multiplex PCR

Quick and simple PCR set-up

We propose ***Hypernova-RED*** DNA Polymerase and ***2x PCR Hypernova-RED*** to simplify PCR operations, especially vital for routine applications e.g. diagnostic assays.

Hypernova-RED DNA Polymerase

Hypernova-RED DNA Polymerase consists of *Hypernova* DNA polymerase with an inert red dye to facilitate accurate low volume pipetting and as an indicator of an enzyme addition. This dye has no adverse effect on the outcome of PCR; yields are the same as with standard *Hypernova* DNA polymerase. Use of the *Hypernova-RED* DNA Polymerase decreases the risk of making a mistake during a reaction set-up e.g. skipping the polymerase, inaccurate reagents mixing. Additionally PCR product can be applied directly onto gel after amplification without mixing with loading buffer.

→ **Facilitate PCR reaction set-up**
→ **Reduced risk of mistake thanks to inert red dye**
→ **Direct gel loading**

2x PCR Hypernova-RED – PCR Mix

The *2x PCR Hypernova-RED* contains *Hypernova* DNA polymerase, dNTPs, optimal PCR buffer and all other components required for PCR, except for DNA template and primers. The *2x PCR Hypernova-RED* application reduces the number of pipetting steps and contamination risk, facilitates greater efficiency, throughput and reproducibility. The PCR mixtures prepared with *2x PCR Hypernova-RED* can be applied onto a gel directly after amplification, no loading buffer use needed. It saves time and facilitates the agarose or polyacrylamide gel electrophoresis preparation.

→ **Facilitate PCR reaction set-up**
→ **Reduced risk of contamination**
→ **Direct gel loading**

LongNova DNA Polymerase

LongNova DNA Polymerase is a blend of *Pwo* and *Taq* polymerases. The thermostable ***LongNova*** DNA Polymerase catalyses DNA synthesis in a 5'→3' directions and shows 3'→5' exonuclease activity. It is characterised by high processivity and proofreading properties. It is ideal for long range PCR amplifications – up to 20 kb.

Features:
- → Wide range of product sizes – from 2 to 20 kb
- → High proofreading properties (3'–5' exonuclease activity)

Applications:
- → Long range PCR
- → Molecular cloning
- → Site-directed mutagenesis and other methods which require high fidelity

Ordering information			
PRODUCT	CONCENTRATION	VOLUME	CAT. NO.
LongNova DNA Polymerase	2 U/µl	200 U	RP281
		1000 U	RP282
LongNova-RED DNA Polymerase	1 U/µl	200 U	RP281R
		1000 U	RP282R
2x PCR LongNova-RED	0.04 U/µl	100 reactions	RP85L
		1000 reactions	RP85L-10

PCR Enhancers

PCR Anti-inhibitor

The *PCR Anti-inhibitor* is a carefully composed mixture of alkaline proteins counteractive to various substances inhibiting the PCR reaction. Addition of the *PCR Anti-inhibitor* to a reaction mixture is an ideal way to eliminate the inhibitors derived from isolation process of a DNA used as a template for PCR. The *PCR Anti-inhibitor* should be added in 1:50 volume ratio to the PCR mixture.

Ordering information		
PRODUCT	VOLUME	CAT. NO.
PCR Anti-inhibitor	100 reactions	RP50
	500 reactions	RP51
TaqSSB Protein	50 µg	RP30
	250 µg	RP305
TneSSB Protein	50 µg	RP31-05
	250 µg	RP31-25
TmaSSB Protein	50 µg	RP32-05
	250 µg	RP32-25
5x GC-Additive	1 ml	RP516
	5x 1ml	RP517

Application
The *PCR Anti-inhibitor* is recommended for PCR with so called difficult DNA templates isolated from the specimen such as urine, saliva, sputum, blood, cell swabs, cerebrospinal fluid, biopsy specimen etc.
M – DNA Ladder M100-500
K- – PCR negative control
S1 – S5 – amplification results obtained for reaction mixtures containing following SDS (PCR inhibitor) concentrations: S1: 0.1%; S2: 0.04%; S3: 0.02%; S4: 0.01%; S5: 0.005%
K+ – PCR positive control (mixture does not contain SDS)
A1 – A2 – amplification results obtained for mixtures containing 0.02% SDS and the following quantities of ***PCR Anti-inhibitor*** (reaction volume 50 µl): A1: 5 µl; A2: 1 µl
A3 – A4 – amplification results obtained for mixtures containing 0.04% SDS and the following quantities of ***PCR Anti-inhibitor*** (reaction volume 50 µl): A3: 5 µl; A4: 1 µl

Thermostable Single-Stranded DNA Binding Proteins (SSB)

TaqSSB – PCR enhancer

Thermostable SSB protein isolated from *Thermus aquaticus*, recommended for general use with techniques requiring extremely high temperature conditions, such as nucleic acid amplification (PCR) and DNA sequencing.

Extreme Thermostable SSB proteins (*TneSSB, TmaSSB*)

Extreme Thermostable Single-Stranded DNA Binding proteins isolated from the hyperthermophilic bacteria *Thermotoga neapolitana* (*TneSSB*) and *Thermotoga maritima* (*TmaSSB*). Due to the extreme thermostability, *TneSSB* and *TmaSSB* can be used in molecular biology applications that require extremely high temperature conditions, such as nucleic acid amplification and sequencing.

Thermostability

TneSSB is the most thermostable SSB protein identified to date, with a melting temperature (Tm) of 112.5°C (Tm of *TmaSSB* is 109.3°C and of *TaqSSB* is 86.8°C). The half-live of the ssDNA-binding activity at 100°C is 12 h (*TmaSSB* is 10 h).

Remaining SSB proteins (from *Thermus thermophilus*, *Deinocossus radiopugnans*, *D. geothermalis*, *D. murrai*) are produced upon request.

Applications of thermostable SSB proteins:

- → Prevents PCR inhibition
- → Increases amplification efficiency
- → Increases selectivity and specificity of multiplex PCR
- → Protects single stranded DNA from degradation
- → Reduces secondary structure formation, which inhibits PCR
- → Enhances amplification of difficult templates (e.g. rich in GC)
- → Stimulates fidelity and processivity of *Taq* polymerase
- → Reacts with RNA allowing increase of a synthesized cDNA size
- → Stabilises ssDNA in the site-specific mutagenesis
- → Facilitate in obtaining complete digestion by restriction endonucleases

5x GC-Additive

The PCR amplification of GC-rich DNA is often problematic due to the stable secondary structures of the DNA, which have high melting temperatures. These secondary structures interrupt DNA polymerase continuous movement along the DNA strand during the polymerization process, resulting in incomplete and nonspecific amplification. Many different methods and additives have been developed to facilitate template denaturation. The *5x GC-Additive* is an ideal tool for amplification on GC-rich templates. It changes the DNA behaviour upon heating and can be used with primer template – pairs with GC-rich content that do not work well with standard PCR conditions. The *5x GC-Additive* reduces the number of secondary structures and enables specific hybridization of primers. It is recommended to be used with *TaqNova* DNA Polymerase to obtain the most satisfactory results.

- → **GC-rich templates**
- → **Ideal for problematic templates, which fail with standard *Taq* DNA polymerases**

We own a substantial collection of thermostable single-stranded DNA-binding (SBB) proteins – PCR enhancers

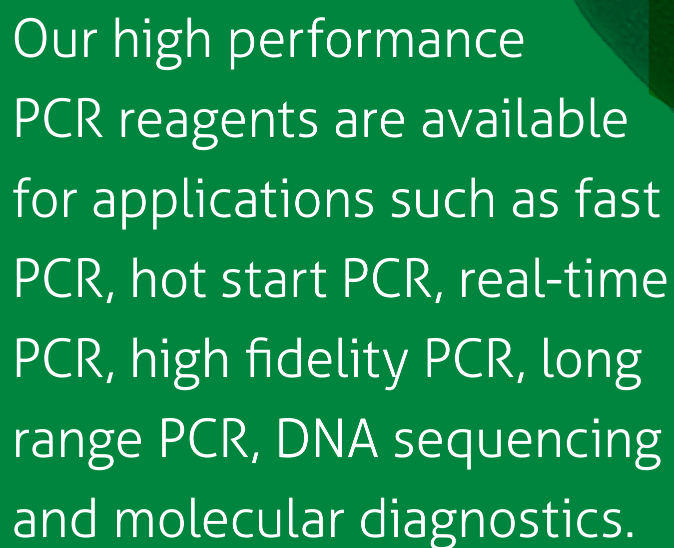
dNTPs

Buffered solutions of ultrapure deoxyribonucleotides (dATP, dCTP, dGTP, dTTP) in various concentrations are ready to use in PCR, RT-PCR and Real-Time PCR reactions. The deoxiribonucleotides are a key component of an amplification reaction and their chemical purity is vital for the satisfactory results. They are supplied as lithium salts, which ensure prolonged stability and greater resistance to repeated freezing and thawing cycles. Thorough mixing of all 4 dNTPs guarantees high amplification efficiency and prevents incorrect incorporation of dNTPs in the synthetized strand.

Properties:

- → Highest purity appointed by an enzymatic synthesis and a high resolution HPLC (>99% dNTP, <0.9% dNDP)
- → Increased sensitivity (allows template detection even in a very low copy number)
- → High yield (DNase-, RNase-, ATPase- and pyrophosphatase-free)
- → Effective and efficient PCR amplification even of very long amplicons (>10 kb)
- → Lithium salt dNTP guarantees high stability and greater resistance to repeated freezing and thawing cycles
- → Free of dNTPs with modified bases and tetra- and pyrophosphates impurities (common PCR inhibitors)

Ordering information			
PRODUCT	CONCENTRATION	VOLUME	CAT. NO.
Equimolar mixtures of dATP, dCTP, dGTP, dTTP			
dNTPs MIX 8 mM Total	2 mM of each dNTP	1 ml	RP61
dNTPs MIX 10 mM Total	2,5 mM of each dNTP	1 ml	RP63
dNTPs MIX 40 mM Total	10 mM of each dNTP	1 ml	RP64
dNTPs MIX 100 mM Total	25 mM of each dNTP	1 ml	RP65
Sets of separate dATP, dCTP, dGTP and dTTP solutions			
dNTPs SET 10 mM	10 mM of each dNTP (separately)	4x 1 ml	RP665
dNTPs SET 100 mM	100 mM of each dNTP	4x 1 ml	RP675


Our high performance
PCR reagents are available
for applications such as fast
PCR, hot start PCR, real-time
PCR, high fidelity PCR, long
range PCR, DNA sequencing
and molecular diagnostics.

Blirt S.A., DNA-Gdańsk
Trzy Lipy 3/1.38, 80-172 Gdańsk, Poland
www.dnagdansk.com | info@dnagdansk.com
T: +48 58 739 61 50 | F: +48 58 739 61 51