# PCR Reagents

blirt DNA GDAŃSK

## *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 GC-reach templates 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 (***TaqNova***, ***Hypernova***, ***LongNova***) along with reaction buffers and magnesium salts for reaction optimization,
- **dNTPs** available as a 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 protein**, **5x GC-Additive** etc.),
- ready-to-use PCR master mixes containing all the necessary PCR reagents in a single tube.

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

| | PRODUCT | 5' –> 3' exonuclease activity | 3' –> 5' exonuclease activity | *Taq* polymerase | *Pyrococcus* polymerase | Proofreading | Mixes | Diagnostic PCR | Long Range PCR (up to 20 kb) | Blunt End Clonning | TA Clonning | GC-rich templates | Difficult templates | Multiplex PCR | Site-directed mutagenesis | Direct load |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| POLYMERASES | *TaqNova* | ✓ | | ✓ | | | ✓ | ✓ | | | ✓ | | | ✓ | | |
| | *TaqNova-RED* | ✓ | | ✓ | | | ✓ | ✓ | | | ✓ | | | ✓ | | ✓ |
| | *TaqNovaGC* | ✓ | | ✓ | | | | ✓ | | | ✓ | ✓ | ✓ | | | |
| | *Hypernova* | | ✓ | | ✓ | ✓ | ✓ | ✓ | | ✓ | | | ✓ | ✓ | ✓ | |
| | *Hypernova-RED* | | ✓ | | ✓ | ✓ | ✓ | ✓ | | ✓ | | | ✓ | ✓ | ✓ | ✓ |
| | *LongNova* | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | | | ✓ | ✓ | |
| | *LongNova-RED* | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | | ✓ | | | ✓ | ✓ | ✓ |
| ENHANCERS | *PCR Anti-inhibitor* | | | | | | | | | | | | ✓ | ✓ | | |
| | *TaqSSB Protein* | | | | | | | | | | | ✓ | ✓ | ✓ | | |
| | *5x GC-Additive* | | | | | | | | | | | ✓ | ✓ | ✓ | | |

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# *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
- Recombinant enzyme of high purity
- Extreme yield with minimal amounts of enzyme and little optimisation
- Half-life of the enzyme is 45 minutes at 94°C
- Amplifies fragments of up to 5 kb
- Leaves ´A´ overhangs

**Applications:**

- Efficient amplification of short and medium size DNA sequences
- Diagnostic PCR

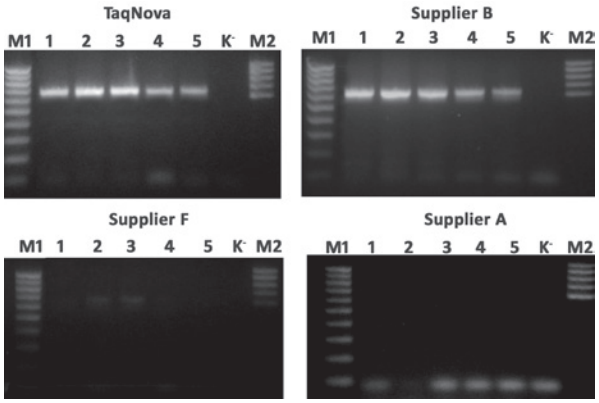



***Fig. 1* Efficiency and sensitivity of Taq polymerases.**
A 630 bp fragment of *ccr5* gene was amplified using the *TaqNova* polymerase and results were compared with the results obtained in a parallel reaction using *Taq* polymerases from supplier B, supplier F and supplier A. The process used serial 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 markers 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**

**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 |
| ***TaqNovaGC*** DNA Polymerase | 5 U/µl | 200 U | RP70-020 |
| | | 1000 U | RP70-100 |

## 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 (over 10 kb). Such long amplicons are often difficult to generate with a single DNA polymerase. *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.

**Advantages:**

- High processivity (for very 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 mixuture 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 characterized 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 PCR products
- → Molecular cloning
- → Site-directed mutagenesis and other methods which require high fidelity

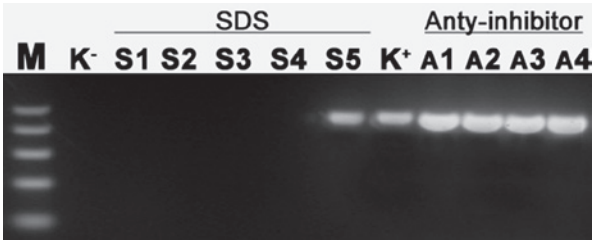
| Ordering information | | | |
|---|---|---|---|
| **PRODUCT** | **CONCENTRATION** | **VOLUME** | **CAT. NO.** |
| ***LongNova*** DNA Polymerase | 2 U/µl | 200 U | RP281 |
| | | 1000 U | RP282 |
| ***LongNova-RED*** DNA Polymerase | 1 U/µl | 200 U | RP281R |
| | | 1000 U | RP282R |
| ***2x PCR LongNova-RED*** | 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 |
| ***5x GC-Additive*** | 1 ml | RP |
| | 5x 1ml | RP |




**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

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

## *TaqSSB protein*

A thermostable protein isolated from *Thermus aquaticus* binding single stranded DNA.

**Applications:**

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

**Note!** We own a substantial collection of the SSB proteins. Remaining SSB proteins (from *Thermus thermophiles*, *Deinocossus radiopugnans*, *D. geothermalis*, *D. murrai*, *Thermotoga neapolitana* and *T. maritime*) are produced upon request.

## *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**
- → **High specifity**
- → **Ideal for problematic templates, which fail with standard *Taq* DNA polymerases**

# 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 kbp)
- → 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 0,2 ml | RP66 |
| dNTPs SET 10 mM | 10 mM of each dNTP (separately) | 4x 1 ml | RP665 |
| dNTPs SET 100 mM | 100 mM of each dNTP | 4x 0,2 ml | RP67 |
| dNTPs SET 100 mM | 100 mM of each dNTP | 4x 1 ml | RP675 |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