

2

2.4 Nucleic Acid Amplification Reagents

137 PCR enzyme selection guide

138 Diamond *Taq*[®] Family

140 Diamond & Red Diamond *Taq*[®]

141 Hot Diamond *Taq*[®]

142 HGS Diamond *Taq*[®]

143 DAp GoldStar[®] DNA polymerase

144 SilverStar DNA polymerase

145 AccuStar[™] DNA polymerase & Booster Mix

146 Authentik[™] DNA polymerase & *Tth* DNA polymerase

147 Ready-to-use PCR Mixes

148 Reverse transcriptase

149 Nucleotides



DNA Polymerases

For optimal amplification and gene analysis results, you need products you can rely on.

Eurogentec provides the best range of PCR enzymes, validated against competitors by top laboratories and major diagnostic companies.

All our DNA polymerase enzymes are purified according to special multiple purification protocols minimizing the amount of trace *E. coli* DNA present in the final *Taq* polymerase preparation, allowing you to work with minimal background. All our enzymes are tested extensively for the absence of nicking and priming activities, exonucleases and non-specific endonucleases.

PCR Enzyme Selection Guide

	Free Sample	PCR	qPCR	Yield	Sensitivity Low copy template	Fidelity	Specificity	Visual recognition	Difficult template (GC & AT rich)	Long template	3' end
Recombinant <i>Taq</i>[®]											
Diamond <i>Taq</i> [®]	TAQ-I021-100	***	*	++	++	++	++		*	**	3' A
Red Diamond <i>Taq</i> [®]	TAQ-I041-100	***	*	++	++	++	++		*	**	3' A
SilverStar	ME-0074-SA	***	*	+++	+++	+	+		*	**	3' A
Accustar	ME-0067-SA	***	*	++	+	+++	++		*	*	Blunt
Dap GoldStar	ME-0068-SA	***	*	++	++	++	++		***	***	3'A + Blunt
Hotstart <i>Taq</i>[®]											
Hot Diamond <i>Taq</i> [®]	TAQ-I033-100	***	**	+++	+++	+	+++		***	***	3' A
Red Hot Diamond <i>Taq</i> [®]	TAQ-I035-100	***	**	+++	+++	+	+++		***	***	3' A
Will be available on June 2011											
HGS Diamond <i>Taq</i> [®]	TAQ-I011-100	***	***	+++	+++	+	+++		**	**	3' A
Authentik DNA polymerase	ME-0070-SA	***	**	+++	+++	++	+++		**	**	3'A + Blunt

* Weakly recommended ** recommended *** Highly recommended
+ Low ++ medium +++ high

Dispensing services



- ❖ Receive your assay pre-dispensed
- ❖ Simplify procedures and reduce workload
- ❖ Improve assay performances
- ❖ Reduce contamination risks
- ❖ Ease accreditation processes

Automate your routine assays to perfection, with full design and format flexibility.

Combine all required reagents, in any type of formulation, support and packaging, to produce your ready & easy-to-use solution.

Our dispensing service is fully scalable and can be adapted to any requirement, for all types of use.

Ask for your free project analysis or request more info at :

dispensing@eurogentec.com

Diamond Taq® Family

The three major GoldStar® enzymes are now manufactured according to fully compliant cGMP processes, purifications and quality controls.

These enzymes have been included into “Diamond Taq® family”:

- GoldStar® ➔ **Diamond Taq®**
- RedGoldStar ➔ **Red Diamond Taq®**
- HotGoldStar ➔ **HGS Diamond Taq®**

General benefits for Diamond Taq® family (due to the manufacturing upgrade)

- Ultra-low residual DNA content
 - ➔ QC-tested ensuring less than 1 fg (0.2 copy) of genomic *E. coli* DNA / Taq unit
- Ultra-low bioburden
 - ➔ Production in a GMP-Pharma facility leading to a guaranteed bioburden between 0 and 10 CFU/ml.
- Higher lot to lot consistency
 - ➔ Strickly monitored GMP and analytical processes ensuring lot-to-lot consistency.
- Higher Sensitivity
- Lower risk of false positive results due to residual DNA contamination (bacterial & fungal).

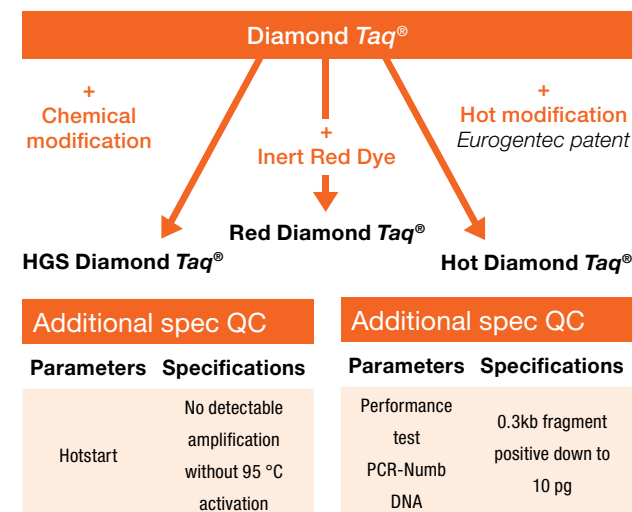
Now also recommended for diagnostic and stringent PCR & qPCR applications. Manufacturing processes are fully compliant to the ISO13485:2003 Medical Device standard.

Diamond Taq® family specifications

Diamond Taq® is a highly thermostable enzyme produced and purified from recombinant *Escherichia coli* bacteria containing the *Thermus aquaticus* DNA Polymerase gene. This thermophilic eubacterium strain lacks Taq I restriction endonuclease. The expressed enzyme shows very good fidelity and catalyzes 5'→3' synthesis of DNA with no detectable 3'→5' proof reading exonuclease activity. The enzyme has the “extendase” activity allowing TA cloning.

Diamond Taq® family specifications

Parameters	Specifications
Appearance	Clear, colorless solution
Identity (SDS-PAGE)	MW approx. 95 kDa
Volume activity	5 U/μl
Purity (SDS-PAGE)	> 98 %
Performance test: PCR - λ DNA	0.5 kb fragment positive down to 5 pg
Performance test: PCR - genomic DNA	0.1 kb fragment positive down to 5 pg
Ribonucleases (up to 10 U, 1 h, 37 °C)	Not detectable
Endonucleases (up to 30 U, 16 h, 65 °C)	Not detectable
Exonucleases (up to 30 U, 16 h, 65 °C)	Not detectable
Nicking activity (up to 30 U, 16 h, 65 °C)	Not detectable
<i>E. coli</i> residual DNA	< 1 fg / Taq Unit
Bioburden	≤ 10 CFU/ml
Stability	24 months (at -20 °C) from date of manufacture
Animal-derived additives	None
Red Solutions for Red Diamond Taq®	



Quality

Our Quality Management System is fully compliant with the ISO 13485:2003 quality standard and FDA's QSR (21 CFR 820). Our GMP facilities incorporate a secure card-key system for access and permit full segregation of production, purification and fill & finish processes.



The entire process is performed under controlled conditions; in class 10 000 clean room for purification (ISO 7 with < 100 cfu/m³) and in class 100 laminar flow hoods for fill & finish (ISO 5 in with < 1 cfu/m³). Final product is released by our Quality Assurance Department with a formal Certificate of Analysis. Full batch records are maintained and can be reviewed during customer or regulatory audits.

Professional support for manufacturers of diagnostic assays

Choosing a manufacturing partner early in the assay development process is an important step towards successful assay validation and product commercialization. Eurogentec's ability to provide a complete custom solution makes the difference.

Experience.

- Over 23 years experience in Oligonucleotide synthesis
- Over 16 years experience in GMP protein manufacturing
- Fully trained and skilled staff
- Expert synthesis & purification chemists

Quality commitment.

- Organization-wide QMS implementation
- Comprehensive risk analysis and mitigation
- Classified clean room facility

Regulatory compliance

- Full compliance to ISO 13485:2003
- Full compliance to FDA cGMP/QSR (21 CFR Part 820)
- EU IVD Directive 98/79 EC compliance

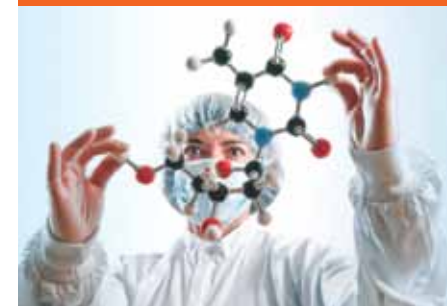
Flexibility - Complete custom solutions

- CMO for manufacturing of bulk reagents
- Fill & Finish, labeling & packaging
- Assay set assembly
- GMP Oligonucleotides and mastermixes
- Collaborative project development

Every aspect of the manufacturing process is monitored and documented providing our partners a level of confidence in quality and regulatory compliance virtually unparalleled in the industry.

Eurogentec has a customer-proven track record of success in delivering our partner's mission critical, GMP-assay components on time, every time guaranteed!

cGMP Oligonucleotides



From first contact to mature projects, we consider each request with utmost priority.

A dedicated project manager and technical specialists ensure perfect coordination between our customer and the production platform to efficiently convert their project into ready and easy-to-use solutions fulfilling all their specific requirements.

Contact info :
info@eurogentec.com

Diamond & Red Diamond Taq®

GoldStar® & RedGoldstar Taq® DNA Polymerases, originally supplied for Research applications, are now manufactured according to a GMP Process and have been renamed Diamond Taq® and Red Diamond Taq® improving their suitability towards *in vitro* Diagnostic & demanding Research

Key benefits

- Same enzyme as the extensively validated GoldStar®.
- Customized Fill & Finish, on request Diamond Taq® enzyme can be produced with:
 - ➔ An activity from 5 to 200 U/μl
 - ➔ A glycerol level from 1 to 50 %
- Visual confirmation of pipetting (inert red dye) included in the storage buffer of Red Diamond Taq®,

The red dye does not influence the PCR reaction and has no effect on automated or manual sequencing, restriction digestions or other downstream applications. Removing the dye can be easily accomplished using any standard purification method.

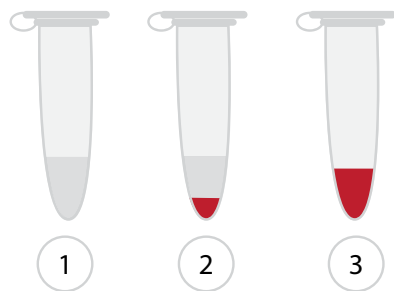


Figure 1: Quick recognition and confirmation of appropriate mixing.

- Vial 1 contains no Red Diamond Taq®
- Vial 2 contains Red Diamond Taq® not mixed, and the vial 3 contains Red Diamond Taq® thoroughly mixed

Additional information

Package content

Diamond Taq® & Red Diamond Taq® are provided at a concentration of 5 U/μl with 10x reaction buffer & MgCl₂ solution.

- 10x Reaction buffer: 750 mM Tris-HCl pH 8.8 (at 25 °C), 200 mM (NH₄)₂SO₄, 0.1 % (v/v) Tween 20 & stabilizer.
- MgCl₂ solution: 25 mM MgCl₂
- Enzyme storage buffer: 20 mM Tris-HCl, 1mM DTT, 0.1 mM EDTA, 0.1 M KCl, 0.5 % Nonidet P40 (v/v), 0.5 % Tween 20 (v/v), 50 % glycerol (v/v) pH 8.0 (4 °C) and stabilizer.
- + inert red dye (for Red Diamond Taq®).

Storage conditions

Storage at -20 °C is recommended.

Documentation

Enzymes are provided with specification sheet & Certificate of Analysis with the QC data released by a QC authorized person and based on review of the complete batch record.

Order & Shipping conditions

Diamond Taq® & Red Diamond Taq® can be ordered & shipped following two processes:

IVD process

Order to diagnostic.taq@eurogentec.com

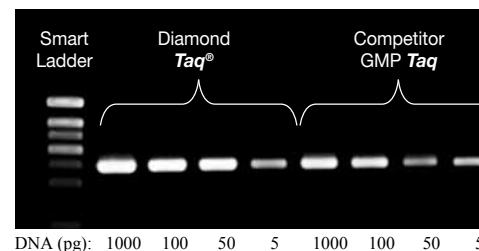
- Shipping on dry ice
- Full traceability from production, storage to shipment of the product
- Tracking number sent to customer the day of the shipment.

Classical process

Order to order@eurogentec.com

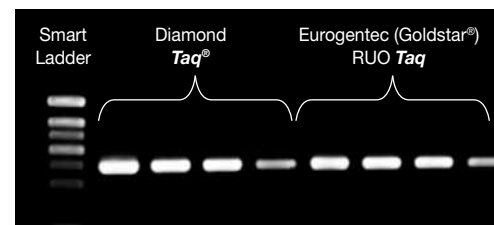
- Shipping at room temperature
- Full traceability from production to storage of the product

Performance test



DNA (pg): 1000 100 50 5 1000 100 50 5

Diamond Taq® compared with competitor GMP Taq®. Diamond Taq® was evaluated against competitor GMP Taq® for its ability to amplify different amounts (from 5 to 1000 pg) of a 0.5 kb PCR template (λ DNA). PCR products were analyzed by gel electrophoresis.



DNA (pg): 1000 100 50 5 1000 100 50 5

Diamond Taq® compared with GoldStar Taq®

Diamond Taq® was evaluated against Eurogentec's GoldStar Taq® for its ability to amplify different amounts (from 5 to 1000 pg) of a 0.5 kb PCR template (λ DNA). PCR products were analyzed by gel electrophoresis.

Process	Diamond Taq®	Red Diamond Taq®
IVD process	TAQ-I020-XXX	TAQ-I040-XXX
Classical process	TAQ-I021-XXX	TAQ-I041-XXX

xxx refers to the unit amount.

Hot Diamond *Taq*[®]

This enzyme, derived from the Diamond *Taq*[®] exhibits unique characteristics according to an exclusive new Hot Start concept.

Hotstart characteristics preventing non-specific polymerisation are accomplished through a proprietary agent, not via a chemical modifications nor a blocking antibody; this prevents primer-dimer formation and increases the PCR yield of specific products. Hot Diamond *Taq*[®] shows no amplification at room temperature and gives very high yield of specific product. The enzyme needs very short activation time (100 % activated during the first PCR cycle) but is compatible with all existing protocols (from 20 sec. to 15 minutes at 95 °C).

Key benefits

- Universal, amplification of long & difficult templates.
- Specific, prevents non specific polymerisation as primer-dimer formation and increases the PCR yield of specific products.
- Very short activation time (minimum 20 sec.)

Process	Hot Diamond <i>Taq</i> [®]
IVD process	TAQ-I032-XXX
Classical process	TAQ-I033-XXX

xxx refers to the unit amount.

Note: Red Hot Diamond *Taq*[®] (TAQ-I035-XXX), including an inert Red Dye will be available in June 2011.

Additional information

Package content

Hot Diamond *Taq*[®] is provided at a concentration of 5 U/μl with 10x reaction buffer & MgCl₂ solution.

- 10x Reaction buffer: 750 mM Tris-HCl pH 8.8 (at 25°C), 200 mM (NH₄)₂SO₄, 0.1 % (v/v) Tween 20 & stabilizer.
- MgCl₂ solution: 25 mM MgCl₂
- Enzyme storage buffer: 20 mM Tris-HCl, 1mM DTT, 0.1 mM EDTA, 0.1 M KCl, 0.5 % Nonidet P40 (v/v), 0.5 % Tween 20 (v/v), 50 % glycerol (v/v) pH 8.0 (4 °C) and stabilizer.

Storage conditions

Storage at -20 °C is recommended.

Documentation

Enzymes are provided with a specification sheet & a Certificate of Analysis with the QC data released by a QC authorized person and based on review of the complete batch record.

Order & Shipping conditions

Hot Diamond *Taq*[®] can be ordered & shipped following two processes:

IVD process

Order to diagnostic.taq@eurogentec.com

- Shipping on dry ice
- Full traceability from production, storage to shipment of the product
- Tracking number sent to customer the day of the shipment.

Classical process

Order to order@eurogentec.com

- Shipping on dry ice
- Full traceability from production to storage of the product

Performance test

Hot Diamond *Taq*[®] is a universal polymerase that efficiently amplifies difficult templates of DNA.



Performance test on difficult template (gDNA NUMB - 306b):

Hot Diamond *Taq*[®] (HDT) & Diamond *Taq*[®] (DT) were evaluated against Hotstart competitors (C1, 2 & 4 - Chemical modification ; C3 - Antibody) for their ability to amplify a difficult PCR template of 0.3 kb. PCR products were analyzed by gel electrophoresis.



Performance test on 72 % GC rich template (SCO3449 - 152b):

Hot Diamond *Taq*[®] (HDT) & Diamond *Taq*[®] (DT) were evaluated against Hotstart competitors (C1, 2 & 4 - Chemical modification ; C3 - Antibody) for their ability to amplify a GC rich PCR template of 0,15 kb. PCR products were analyzed by gel electrophoresis.

HGS Diamond *Taq*[®]

HotGoldStar *Taq*[®] DNA Polymerase, originally supplied for Research applications only, is now manufactured according to a GMP Process and has been renamed HGS Diamond *Taq*[®] improving its suitability towards *in vitro* Diagnostic & demanding Research applications.

HGS Diamond *Taq*[®] is a chemically modified HotStart *Taq*[®] DNA polymerase, which completely lacks any activity below 74 °C. This avoids non-specific priming at low temperature without a thermal activation of 5 minutes at 95 °C to reach maximal initial activity. During the PCR the rest of the enzyme activity is released. HGS Diamond *Taq*[®] is more heat-stable than commonly used *Taq*[®] DNA polymerase.

DNA fragments as long as 2 kb can be efficiently amplified. HGS Diamond *Taq*[®] DNA polymerase provides efficient amplification of specific products without amplifying non-specific products or primer dimers.

Key benefits

- ▣ Specific, prevents non specific polymerisation as primer-dimer formation and increases the PCR yield of specific products.
- ▣ Proven performance in many applications, same enzyme as extensively validated and benchmarked HotGoldStar.
- ▣ Customized Fill & Finish, on request HGS Diamond *Taq*[®] enzyme can be produced with:
 - ⇒ An activity from 5 to 50 U/μl
 - ⇒ A glycerol level from 1 to 50 %

Additional information

Package content

HGS Diamond *Taq*[®] is provided at a concentration of 5 U/μl with 10x reaction buffer & MgCl₂ solution.

- ▣ 10x Reaction buffer: 150 mM Tris-HCl pH 8.5 (at 25°C), 500 mM KCl & stabilizer.
- ▣ MgCl₂ solution: 25 mM MgCl₂

Storage conditions

Storage at -20 °C is recommended.

Documentation

Enzymes are provided with a specification sheet & a Certificate of Analysis with the QC data released by a QC authorized person and based on review of the complete batch record.

Order & Shipping conditions

Hot Diamond *Taq*[®] can be order & shipped following two processes:

IVD process

Order to diagnostic.taq@eurogentec.com

- ▣ Shipping on dry ice
- ▣ Full traceability from production, storage to shipment of the product
- ▣ Tracking number sent to customer the day of the shipment.

Classical process

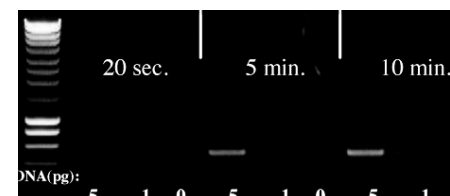
Order to order@eurogentec.com

- ▣ Shipping at room temperature
- ▣ Full traceability from production to storage of the product

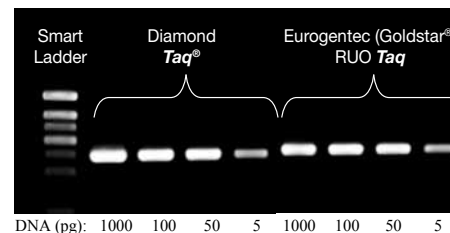
Process	HGS Diamond <i>Taq</i> [®]
IVD process	TAQ-I010-XXX
Classical process	TAQ-I011-XXX

xxx refers to the unit amount.

Performance test



HGS Diamond *Taq*[®] is a hotstart chemically modified that shows no amplification at room temperature and gives very high yield of specific product. The enzyme needs a minimum of 5 minutes of activation at 95 °C.



HGS Diamond *Taq*[®] compared with Hot GoldStar[®] *Taq*.

HGS Diamond *Taq*[®] was evaluated against Eurogentec's HotGoldStar (HGS) for its ability to amplify in triplicate 5 pg of a 0.5 kb PCR template (λ DNA). PCR products were analyzed by gel electrophoresis.

DAP GoldStar® DNA polymerase

DAP (Difficult Amplification) GoldStar® is a high-performance proprietary complex of enzymes and additives, specially designed for applications requiring high processivity and high yields. It is recommended for difficult, long, and/or impure fragments. An advanced incubation buffer is supplied for optimal results and enhance enzyme life. Moreover an additive is provided to improve specificity of the reaction, especially on GC-rich or difficult templates. DAP GoldStar® has 5'→3' exonuclease and possesses 3'→5' proofreading activity, which prevents misincorporations during extension. It also has the extendase activity allowing TA cloning.

Key benefits

- High processivity (3' to 5' proofreading)
- High yields
- Long range applications up to 30 kb genomic DNA
- > 7 fold higher fidelity than a normal *Taq* polymerase
- Suitable for difficult templates
- Suitable for TA cloning

Applications

- Long region amplifications: using long primers at elevated Mg^{2+} concentrations, > 30 kb or 15 kb products can be achieved from lambda templates or genomic DNA respectively.
- High performance: DAP GoldStar® provides high yield and specificity, even with impure DNA or difficult templates. Unlike standard 3'→5' proofreading polymerases, DAP GoldStar® can be used in combination with primers containing wobbles.

- Direct cloning: DAP GoldStar® is recommended for direct gene TA cloning without the need to verify the sequence prior to expression.
- High Fidelity: DAP GoldStar® is a mix of polymerases that possesses 5'→3' DNA polymerase activity and 3'→5' proofreading activity which prevents misincorporations during primer extension. DAP GoldStar® contains an additive, which eliminates the sequence-dependent removal of 3'-terminal dNMPs in growing DNA chains (pyrophosphoro-lysis). This combination of properties can provide a > 7 fold higher fidelity than *Taq*® DNA polymerase. In contrast with other proofreading enzymes, DAP GoldStar® does not degrade primers.

Note: For longer regions, the addition of glycerol or DMSO to a final concentration of 2 - 8 % may improve performance.

Additional information

Package Content

- DAP GoldStar® DNA polymerase (4 U/μl)
- 10x Optibuffer without $MgCl_2$
- 50 mM $MgCl_2$
- 5x Improving additive
- DMSO

Storage conditions

Storage at -20 °C is recommended.

Shipping conditions

Shipped at -20 °C.

Quality Control

The DAP GoldStar® is tested extensively for activity, non-specific endonuclease / nickase and exonuclease activity.

Unit definition

The amount of enzyme required to incorporate 10 nmol of dNTPs into acid-insoluble material in 30 minutes at 72 °C.

DAP GoldStar® DNA polymerase

Description	Quantity	Reference
DAP GoldStar® DNA polymerase	250 Units	ME-0068-01
	500 Units	ME-0068-05

SilverStar DNA polymerase

SilverStar is a high-performance thermostable recombinant DNA polymerase, isolated from *Thermus aquaticus*. It is an extremely robust Taq DNA polymerase that does not require optimisation and gives high yields with minimal background consistently from one reaction to another. It is a highly active enzyme that produces excellent results across a wide range of applications such as DNA Array.

The SilverStar has a 5'→3' exonuclease activity and also the extendase activity allowing TA cloning. The enzyme lacks the proofreading activity 3'→5' exonuclease.

Key benefits

- ▣ High yields
- ▣ Robust PCR
- ▣ Highly purified Taq polymerase
- ▣ No optimisation needed

Applications

The SilverStar will efficiently amplify products up to 10 kb giving high yields and optimal results for application such as array PCR product constructions.

Additional information

Package content

- ▣ SilverStar DNA polymerase (5U/μl)
- ▣ 10x buffer, 160 mM $(\text{NH}_4)_2\text{SO}_4$, 670 mM Tris-HCl (pH 8.8 at 21 °C), 0.1 % Tween®20
- ▣ 50 mM MgCl_2

Storage conditions

Storage at -20 °C is recommended.

Shipping conditions

Shipped at -20 °C.

Quality Control

It is specially purified so as to remove traces of DNA that could interfere with some reactions and is free from all non-specific endonucleases, nickases and exonucleases.

Unit definition

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72 °C.

SilverStar DNA polymerase

Description	Quantity	Reference
SilverStar DNA polymerase	500 Units	ME-0074-05
SilverStar dilution buffer	1 ml	ME-0000-DI
SilverStar reaction buffer	1.5 ml	ME-0000-RE

Performance test



DNA Array application = 50,000 ORFs from more than 10 different organisms have been successfully amplified.

AccuStar™ DNA polymerase

AccuStar™ DNA polymerase is a thermostable enzyme possessing 5'→3' DNA polymerase and 3'→5' proofreading exonuclease activities, offering extremely high-fidelity (up to 47-fold higher fidelity than *Taq*®). AccuStar™ DNA polymerase produces blunt-ended amplicons of up to 5 Kb in length.

AccuStar™ DNA polymerase is supplied with 10x Reaction Buffer containing MgSO₄, which provides optimal final reaction conditions (2 mM Mg²⁺) for most experiments. In order to allow optimization of reaction conditions, additional MgCl₂ is provided.

The specificity and performance of AccuStar™ DNA polymerase can be further improved with the use of 2x Booster Mix (not supplied), which is designed for GC or AT-rich DNA, “dirty” templates or sequences with difficult melting profiles.

Key benefits

- High-fidelity coupled with high yield
- Amplifies fragments up to 5 Kb
- Ultra-high fidelity for subsequent cloning
- Blunt-end cloning

Additional information

Package content

- AccuStar™ DNA polymerase (2.5 U/μl)
- 10x buffer
- 50 mM MgCl₂

Storage conditions

Storage at -20 °C is recommended.

Shipping conditions

Shipped at room temperature.

Quality Control

It is specially purified so as to remove traces of DNA that could interfere with some reactions and is free from all non-specific endonucleases, nickases and exonucleases.

Unit definition

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 72 °C.

AccuStar™ DNA polymerase		
Description	Quantity	Reference
AccuStar™ DNA polymerase	250 Units	ME-0067-02
	500 Units	ME-0067-05

Performance test



A serial dilution of template was performed to demonstrate the high performance of AccuStar™, even at low DNA concentrations.

Booster Mix

Booster Mix is a special 2x additive for use in reactions involving any thermostable DNA polymerase, and is designed to dramatically improve reaction specificity. Booster mix provides an optimised composition of reagents, and is ideally suited to dirty/difficult templates with GC or AT-rich DNA, repetitive sequences or difficult melting profiles.

Key benefits

- Dramatically improves specificity and yield
- Compatible with all commercially available thermostable DNA polymerases
- Ideal for “difficult” templates and especially designed for the GoldStar®, HotGoldStar, AccuStar™ and Authentik™ DNA polymerases
- Reduces smearing and background
- DNA polymerase reactions where specificity is critical
- Enhancing the performance and specificity of any thermostable DNA polymerase

Activity

Booster Mix acts as a melting agent by facilitating the DNA polymerase and oligonucleotides access to the template DNA. Booster Mix does not contain magnesium, dNTPs, or buffer components. In some cases it may be necessary to optimize the magnesium concentration.

Booster mix		
Description	Quantity	Reference
Booster Mix (2)	2.4 ml	ME-0076-02

Authentik™ DNA polymerase

Authentik™ DNA polymerase is a heat-activated high-performance proprietary complex of enzymes specifically designed for low-copy templates or challenging PCR assays which require both high processivity and high-fidelity. Authentik™ provides improved specificity and very high PCR sensitivity, thereby eliminating the presence of non-specifics such as primer-dimers and mis-primed products. Authentik™ is inactive at room temperature and therefore requires activation by heat treatment for 10 minutes at 95 °C prior to the PCR reaction being performed.

Authentik™ DNA Polymerase is recommended for short Genomic DNA fragments of up to 3 kb, or up to 5 kb on Lambda DNA. Authentik™ is ideal for direct cloning without the need to verify the sequence prior to expression. Authentik™ possesses 5→3' polymerase activity and 3'→5' proofreading activity, which in combination with other properties, provides higher fidelity than *Taq*®. The specificity and performance of Authentik™ DNA Polymerase can be further improved with the use of the Booster Mix (see previous page), which is designed for GC or AT rich DNA sequences.

Key benefits

- ▶ Heat activation for ultra-high specificity
- ▶ High yield coupled with high-fidelity
- ▶ Convenient set up at room temperature
- ▶ Amplifies fragments up to 5 kb
- ▶ Ideal for low-copy or challenging PCR assays
- ▶ Suitable both for TA and for blunt-end cloning

Additional information

Package content

- ▶ Authentik™ DNA polymerase (4 U/μl)
- ▶ 10x buffer
- ▶ 50 mM MgCl₂

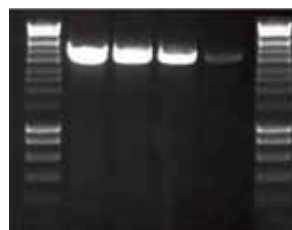
Storage conditions

Storage at –20 °C is recommended.

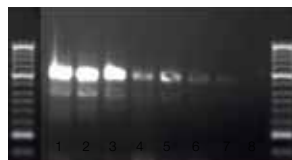
Shipping conditions

Shipped at room temperature.

Performance test



A 2-fold serial dilution of template lambda DNA (5 Kb) was performed from 0.25 ng (lane 1) to 31.25 pg (lane 4)



A 2-fold serial dilution of template human fragment of β actin gene (800 bp) was performed from 50 ng (lane 1) to 390 pg (lane 8)

Amplification from a variety of starting template concentration

Authentik™ DNA polymerase		
Description	Quantity	Reference
Authentik™ DNA polymerase	250 Units	ME-0077-02
	500 Units	ME-0077-05

Tth DNA polymerase

Tth polymerase is a highly thermostable enzyme especially recommended for the amplification of DNA fragments longer than 4 kb. *Tth* DNA polymerase also has a reverse transcriptase activity, making it suitable for RT-PCR.

Additional information

Package content

- ▶ *Tth* DNA polymerase (5 U/μl)

Storage conditions

Storage at –20 °C is recommended.

Shipping conditions

Shipped at –20 °C.

Quality Control

Tested for the absence of nicking and priming activities of exonucleases and nonspecific endonucleases.

Unit definition

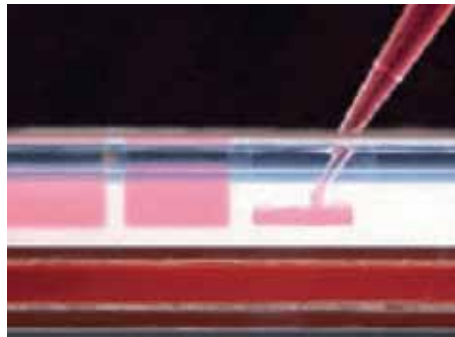
The amount of enzyme that will incorporate 10 nmol of dNTPs into acid-insoluble material in 30 minutes at 75 °C.

<i>Tth</i> DNA polymerase		
Description	Size	Reference
<i>Tth</i> DNA polymerase	100 U	ME-0070-01
	500 U	ME-0070-05

Ready-to-use PCR mixes

Key benefits

- Easy-to-use, 2x concentrated, premixed MasterMixes
- Optimized for a broad range of targets (< 3 Kb)
- Highest sensitivity and yield
- Robust amplification of GC-rich sequences
- Achieves the highest quality sequencing data
- Optionally includes a red loading dye



No loading buffers or tracking dyes required. Samples may be added directly to an agarose gel after PCR without the addition of a loading buffer or tracking dye. The red dye migrates as a 400-500 bp fragment in agarose gel 1 % in TBE buffer.

GoldStar® Mix / Red'y'Gold Mix

2 x concentrated PCR mixes are convenient premixed solutions that include in a single tube all common reagents needed for PCR. Simply dilute twice the PCR mix with template, primers and water to start your reaction. The elimination of cumbersome preparation steps prevents contamination risk by multiple pipetting. Mixes usage also increases the reproducibility of PCR reactions. The Red'y'Gold Mix includes a colored loading buffer allowing the direct loading of the amplicons on a gel.

HotGoldStar Mix / Red'y'Star Mix

For amplifications requiring high specificity use mixes containing the HotGoldStar *Taq*® polymerase, which completely lacks any activity before the activation step, avoiding non-specific annealing at low temperature. The Red'y'Star Mix includes a colored loading buffer allowing the direct loading of the amplicons on a gel.

Ready-to-use PCR mixes

Product	Reference	Quantity	RXNs	Kit Content	Red loading dye
GoldStar Mix 2x	PK-0064-02	5 x 1 ml	200	GoldStar® <i>Taq</i> polymerase (1u/25µl), dNTPs(400 µM), MgCl ₂ (3 mM), Buffer	-
Red'y'Gold Mix 2x	PK-0064-02R	5 x 1 ml	200		✓
HotGoldStar Mix 2x	PK-0073-02	5 x 1 ml	200	HotGoldStar <i>Taq</i> polymerase (1u/25µl), dNTPs(400 µM), MgCl ₂ (3 mM), Buffer	-
Red'y'Star Mix 2x	PK-0073-02R	5 x 1 ml	200		✓

Reverse Transcriptase

Mu-MLV reverse transcriptase

Purified from an overexpressing clone of *E. coli* that carries the Reverse Transcriptase gene from Mu-MLV.

Key benefits

Mu-MLV reverse transcriptase has lower RNase H activity than AMV reverse transcriptase, which is an advantage when synthesizing cDNAs from long mRNAs. Mu-MLV lacks the 3'→5' exonuclease activity. It is recommended to use 10 U/μg RNA for optimum reverse transcription.

Applications

Mu-MLV reverse transcriptase is able to synthesize a DNA strand complementary to an RNA template in the presence of a primer. This enzyme is particularly suited for long fragments.

Additional information

Package content

- Mu-MLV reverse transcriptase (200 U/μl)
- 5x cDNA buffer: 250 mM Tris-HCl (pH 8.3), 375 mM KCl, 50 mM DTT, 15 mM MgCl₂

Storage conditions

Storage at -20 °C is recommended.

Shipping conditions

Shipped at -20 °C.

Quality Control

Tested for the absence of endonucleases and RNases.

Unit definition

The amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble form in 10 minutes at 37 °C using polyA-oligo-dT(12-18) as substrate.

Mu-MLV reverse transcriptase

Description	Size	Reference
Mu-MLV reverse transcriptase	8000 U	ME-0125-80
	40000 U	ME-0125-400

Nucleotides

Purity

dNTPs are enzymatically produced from nucleotide monophosphates by a process of phosphorylation. This process uses highly specific enzymatic systems, which eliminate impurities and PCR inhibitors such as modified nucleotides and pyrophosphates. dNTPs are purified with preparative HPLC and are at least 99 % pure.

Performance

dNTPs offer reliable and reproducible performance in any enzymatic reaction, including PCR and reverse transcription. Owing to Lithium salt preservation, dNTPs are more resistant to repeated freeze/thawing than other commercially available dNTPs, and remain sterile during the entire storage period. These properties are advantageous for laboratory procedures.

Configuration

dNTPs are available as convenient 100 mM sets in three pack sizes or in ready-to-use dNTP mixes, which are designed to save time and reduce the risk of contamination. The dNTP solutions are supplied ready-to-use as Lithium salts in water at pH 7.0.

Lithium salts have greater resistance to repeated freezing and thawing cycles than Sodium salts dNTPs, and remain sterile over the entire shelf-life due to the bacteriostatic activity of Lithium towards various microorganisms.

Quality Control

dNTPs are tested for the absence of DNase, RNase, protease and nicking activity. Each batch is validated rigorously for purity by HPLC, and tested for performance in a wide range of PCR templates, including a long-distance 20 kb functional test.

dNTP mix

Description

dNTP mix containing all four nucleotides (dATP, dCTP, dGTP, dTTP) in a premixed solution, ready for immediate use (5 mM each).

Application

For primer extension technology, add 4 μ l of dNTP mix to 96 μ l reaction mix to give a final concentration of 200 μ M per dNTP.

dNTP MIX (Lithium salts)				
Description	Quantity	Volume	Concentration	Reference
dNTP mix	1 x 20 μ moles	1 ml	4 x 5 mM	NU-0010-10
	5 x 20 μ moles	5 x 1 ml	4 x 5 mM	NU-0010-50
	10 x 20 μ moles	10 x 1 ml	4 x 5 mM	NU-0010-100

dNTP set

Description

A pack of four vials, each containing one of the four nucleotides (dATP, dCTP, dGTP, dTTP).

Application

For primer extension technology, prepare a dNTP mix and dilute to give a final concentration of 200 μ M of each dNTP in a total 100 μ l reaction mix.

dNTP SET (Lithium salts)				
Description	Quantity	Volume	Concentration	Reference
dNTP set	4 x 5 μ moles	4 x 500 μ l	10 mM each	NU-0020-10
	4 x 25 μ moles	4 x 250 μ l	100 mM each	NU-0020-50