

EUROGENTEC HEADQUARTERS

LIEGE SCIENCE PARK • 4102 Seraing • Belgium • Tel.: +32 4 372 74 00
 Fax: +32 4 372 75 00 • info@eurogentec.com • www.eurogentec.com

**EUROGENTEC NORTH AMERICA, INC.**

11111 Flintkote Avenue • San Diego CA 92121-1222 USA
 Tel.: +1 858 793 2661 • Fax: +1 858 793 2666 • info.usa@eurogentec.com
 www.eurogentec.com

Authentik™ DNA polymerase ME-0077-02 • ME-0077-05 • ME-0077-SA

Eurogentec products are sold for research or laboratory use only and are not to be administered to humans or used for medical diagnostics.

Batch details

Units per vial:	ME-0077-SA	50 units
	ME-0077-02	250 units
	ME-0077-05	500 units

Concentration: 4 U/μl

Source

Purified from an *E.coli* which carries a Specific DNA polymerase overproducing Plasmid.

Description

Authentik™ DNA polymerase is a heat-activated high-performance proprietary complex of enzymes specifically designed for low copy 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 performing the PCR reaction.

Package contents

Reagent	Volume	Reference
Authentik™ DNA polymerase	12.5 μl	ME-0077-SA
	62.5 μl	ME-0077-02
	125 μl	ME-0077-05
10x reaction buffer Proprietary Non-Tris incubation buffer	1.2 ml 2x 1.2 ml	ME-0077-02/SA ME-0077-05
50 mM MgCl ₂	1.2 ml	ME-0077-02/05/SA

Shipping conditions

Shipping at ambient temperature has no detrimental effect on the performance of this enzyme (if lower than 35 °C).

Storage conditions

Storage at -20 °C is recommended.

Storage buffer

20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 2 mM DTT, 50 % Glycerol, and stabilizers.

Analysis conditions

25 mM TAPS, pH 9.3 (at 25 °C); 50 mM KCl; 2 mM MgCl₂; 1 mM β-mercaptoethanol; 250 μM each dCTP, dGTP, dTTP; 250 μM (³H) dATP (0.05 Ci/mmol); activated salmon sperm DNA (1.25 μg/μl); total volume of 50 μl.

Associated activities

Endonuclease and exonuclease activities were not detectable after 4 hours of incubation of 1 μg of pBR322 plasmid DNA and 0.5 μg *Hind* III-digested lambda DNA at 72 °C in the presence of 20 units of Authentik™.

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.

Reaction Conditions**For a 50 μl Reaction**

10x Reaction Buffer (provided)	5 μl
50 mM MgCl ₂ Solution (provided)	2-6 μl
dNTPs final concentration	200 μM each dNTP
(20 mM dNTP Mix)	2 μl (see related products)
DNA template	1 ng
Primers	0.1 nmoles
Authentik™ DNA Polymerase	0.5-2 μl
Water (ddH ₂ O)	up to 50 μl

EUROGENTEC HEADQUARTERS

LIEGE SCIENCE PARK • 4102 Seraing • Belgium • Tel.: +32 4 372 74 00
Fax: +32 4 372 75 00 • info@eurogentec.com • www.eurogentec.com



Eurogentec

Experience true partnership

EUROGENTEC NORTH AMERICA, INC.

11111 Flintkote Avenue • San Diego CA 92121-1222 USA
Tel.: +1 858 793 2661 • Fax: +1 858 793 2666 • info.usa@eurogentec.com
www.eurogentec.com

Cycling conditions

Authentik™ Activation	Preheat at 95 °C for 10 minutes
Denaturation	94-97 °C
Annealing	see below
Elongation suggested	40-60 seconds per 1 Kb at 68 °C

Time and temperature for denaturation and annealing steps depend on the type of machine and primers. We advice to check primers design using a primer design software.

NB: The specificity and performance of Authentik™ DNA polymerase can be further improved with the use of our Booster Mix (ME-0076-02), which is designed for GC or AT-rich DNA or sequences with a high level of secondary structure.

Troubleshooting guide

Observation	Recommended solution(s)
Low or No amplification product	<ul style="list-style-type: none">> Increase the amount of enzyme in 0.5 U increments.> Increase the magnesium concentration in 0.25 mM increments with a starting concentration of 1.75 mM.> Titrate primer concentration (0.3-1 µM); ensuring that both primers have the same concentration.> Return reactions to the thermal cycler for 5 more cycles, or repeat the reactions<ul style="list-style-type: none">> Make sure primers are not self complementary or complementary to each other. Verify that primers are complementary to the appropriate DNA sequences. Design new primers to prevent self-annealing.> Try Booster Mix (ME-0076-02) to lower the melting profile and improve performance.
Appearance of a smear	<ul style="list-style-type: none">> Reduce the quantity of polymerase in 0.5 U increments.> Reduce the cycle number by 3-5 to remove non-specific bands.> Reduce Extension time in 0.5-1 minute increments.> Reduce the template concentration

Appearance of contaminating bands	<ul style="list-style-type: none">> Increase annealing temperature. Primer annealing should be at least 5 °C below the calculated Tm of primers.> Try Booster Mix (ME-0076-02), to improve specificity.
-----------------------------------	--

Related products

Reagent	Package size	Reference
dNTP Mix 20mM total	1 X 20 µmol 5 X 20 µmol 10 X 20 µmol	NU-0010-10 NU-0010-50 NU-0010-100
dNTP Set 5mM each dNTP	4 X 5 µmol 4 X 25 µmol	NU-0020-10 NU-0020-50
Booster Mix	2.4 ml	ME-0076-02
HotGoldStar DNA Polymerase	500 units	ME-0073-05
SilverStar DNA Polymerase	500 Units	ME-0074-05

For further information please contact our Customer Help Desk:

For Europe:

E-mail: info@eurogentec.com
Tel: +32 4 372 76 65

For USA:

E-mail: info.usa@eurogentec.com
Tel: +1 858 793 26 61