Research in Molecular Microbiology **No. 2/17: 1-2**

Application Note

Hot MolTaq 16S/18S – DNA-Free Hot Start Taq for Bacterial and Fungal PCR Detection at Low Loads

Keywords: *sterility testing, quality control testing, culture-independent diagnosis, bacteria, fungi, Real-Time PCR, 16S, 18S, 23S, 28S rRNA gene, pan-bacterial, pan-fungal assays*

Marina Linow - Molzym GmbH & Co. KG, Bremen, Germany

Abstract: There is a high demand for ultra-pure PCR reagents, in particular heat stable DNA polymerases, for sensitive assaying of bacteria and fungi at low loads. Applications include sterility testing of health care and pharma products, monitoring of laboratory animals for infections, microbial contamination of food and feed, infection of crops, diagnosis of infectious diseases and other. Quality measures for polymerases like Taq DNA polymerase demand high amplification activity, high target specificity by hot start characters and at the same time very low contamination by microbial DNA. Here, a new Taq DNA polymerase, *Hot MolTaq 16S/18S*, is presented that combines all of the mentioned quality characters and is explicitly useful for bacterial and fungal DNA screening purposes.

Introduction

Bacteria and fungi can be present at very low loads in specimens [1]. Therefore, molecular analysis of microbial DNA often takes place at the limit of assay detection. This is challenging because amplification may be hampered by a variety of parameters which negatively influence the sensitivity and specificity of assays, particularly when primer binding sites are conserved regions within the 16S, 23S, 18S, 28S rRNA genes and their internal transcribed sequences of bacteria and fungi, respectively. Among these negative factors, contamination by microbial DNA of PCR reagents and consequently resulting false positive results is a serious problem [2]. Also, prevention of signals from the amplification of primer dimers and other unspecific binding of primers is desirable. Molzym provides a DNA-free Taq, *MolTaq 16S/18S*, which is well suited for the extremely sensitive detection of bacterial and fungal sequences. A new product is available now, *Hot MolTaq 16S/18S*, which combines these favourable characters with hot start amplification and thereby increased specificity. In this article, a summary of the specifications of *Hot MolTaq 16S/18S* will be presented.

Table 1: *Specifications of Hot MolTaq 16S/18S Taq polymerase for the ultra-sensitive and specific amplification of bacterial and fungal sequences.*

Specifications	
Enzyme	thermostable, DNA-dependent DNA polymerase (EC 2.7.7.7.)
Origin	*Thermus aquaticus*
Application	hot start PCR
Inhibitor	Aptamer-based
Purity	DNA-free
Amplification	up to 40 cycles
Activation step	not required
Enzyme concentration	5U/µl
Standard buffer	1.5mM $MgCl_2$
Optional buffer	magnesium-free
Solutions	100mM $MgCl_2$, DNA-free PCR grade water

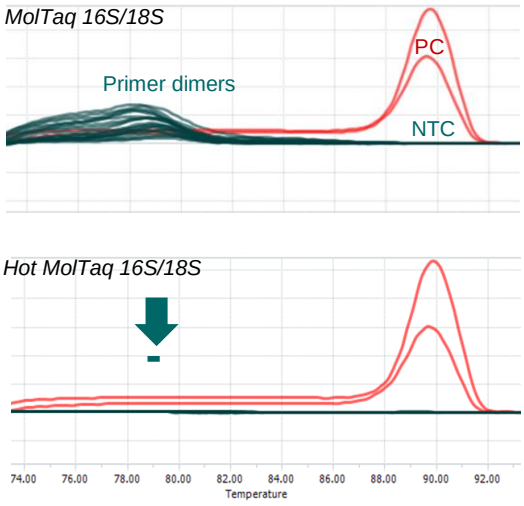

Fig 1: *Comparison of DNA-free, non-hot start MolTaq 16S/18S with DNA-free, hot start Taq, Hot MolTaq 16S/18S. Melt curve analysis of Real-Time PCRs with SYBR Green I. PC: positive controls; NTC: negative controls.*

Suppression of Non-Specific Amplification

Hot MolTaq 16S/18S is an aptamer-stabilised Taq that is inactive at ambient temperature (Table 1) and allows handling without the need for cooling. The enzyme is activated during the initial denaturation phase of the PCR. As a result, products from primer dimer formation are greatly reduced (Fig. 1, arrow, lower graph). The enzyme is supplied with a standard buffer

Contact: Marina Linow, Molzym GmbH & Co. KG, D-28359 Bremen, Germany
Tel.:+49(0)421 696 162 15; e-mail: linow@molzym.com

(1.5mM $MgCl_2$) and a magnesium-free buffer and extra magnesium solution for assay development (Table 1).

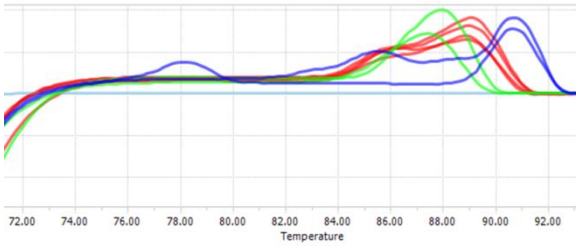

Fig 2: *Hot MolTaq 16S/18S analysis of microbial DNA in sterility testing of a surgery robot (Molzym's 16S rRNA gene PCR assay). Melt curve analysis of Real-Time PCRs with SYBR Green I.*

The beneficial effect of *Hot MolTaq 16S/18S* is demonstrated by an analysis for bacterial sequences of swabs taken from a surgery robot (Fig. 2). Non-hot start *MolTaq 16S/18S* showed some diversity indicated in the range 82 to 92°C. Much more clear signals were noticed with *Hot MolTaq 16S/18S* in the same temperature range (Fig. 2). Apparently, the increased specificity of *Hot MolTaq 16S/18S* revealed unambiguous detection of microorganisms.

High Activity

Standard *MolTaq 16S/18S* is a very active polymerase that at the same time is free of DNA contamination [3]. Figure 3 shows the log-linear function of bacterial DNA amounts/PCR over 5 orders of magnitude against the amplification thresholds (C(t)). The plot shows that *Hot MolTaq 16S/18S* has an activity as high as *MolTaq 16S/18S* allowing detection of bacterial sequences at femtogram amounts.

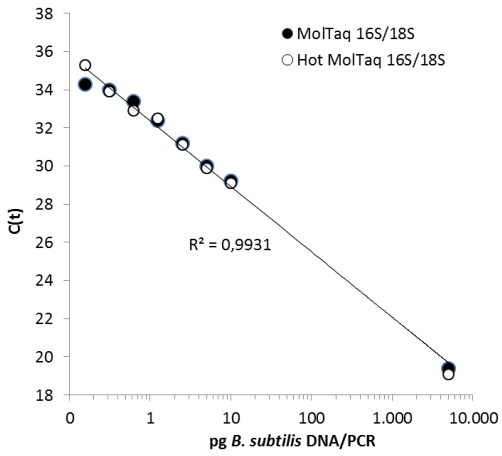

Fig 3: *Plot of threshold values (C(t)) against bacterial DNA input of non-hot start MolTaq 16S/18S and Hot MolTaq 16S/18S. Assay: Molzym Mastermix16S Complete*

Absence of DNA contamination

Molzym's *Hot MolTaq 16S/18S* is routinely tested lot-by-lot for absence of contaminating bacterial and fungal DNA. Negative control reactions are run with DNA-free water added to a master mix with primers that bind to conserved sites of the 16S and 18S rRNA genes of bacteria and fungi, respectively. A gel is depicted in Fig. 4

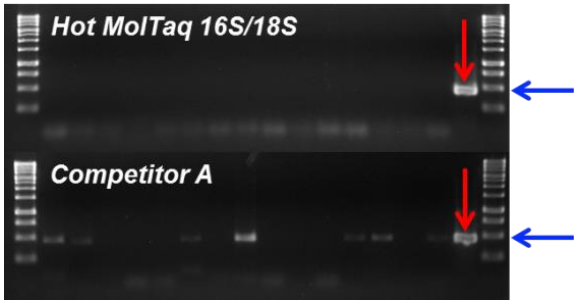

Fig 4: *Negative control testing (water instead of DNA) of Hot MolTaq 16S/18S and low DNA contamination-declared hot start Taq of a competitor for background 16S rRNA gene sequences. Red arrow: positive control; blue arrow: line of amplicon banding (450bp).*

which shows the absence of the 450bp amplicon in 15 runs with *Hot MolTaq 16S/18S*. Activity of the enzyme is indicated in the positive control run with *B. subtilis* DNA (red arrow). In contrast, another Taq (lower image) shows bands of the amplicon (blue arrow) in 7 of the 15 runs. Also, another two Taqs that are declared by the suppliers as decontaminated from bacterial DNA

Table 2: *Comparison of negative control runs of Hot MolTaq 16S/18S Taq polymerase with other Taqs declared as lowly contaminated with bacterial DNA.*

Supplier	Modification	Positive/total reactions
Molzym	aptamer	0/15
Competitor A	chemical	7/15
Competitor B	aptamer	12/15
Competitor C	unknown	14/15

turned out to be contaminated at a considerable rate (Table 2).

Summary

In conclusion, *Hot MolTaq 16S/18S* is a DNA-free hot start Taq DNA polymerase that is ideally suited for the very sensitive and specific PCR and Real-Time PCR analysis of bacterial and fungal DNA at low concentrations.

References

[1] Kellogg JA et al. (2000) J Clin Microbiol 38, 2181-2185.

[2] Lorenz MG (2016) Res Mol Microbiol (newsletter Molzym) 2/16, 1-8.

[3] Mühl H et al. (2010) Diagn Microbiol Inf Dis 66, 41–49.

Contact: Marina Linow, Molzym GmbH & Co. KG, D-28359 Bremen, Germany
Tel.:+49(0)421 696 162 15; e-mail: linow@molzym.com