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Direct Oral Swab 2x PCR Master Mix

#9501
Store at -20°C

Contents

Direct Oral Swab 2x PCR Master Mix contains all components necessary for direct PCR or PCR based genotyping directly from saliva or buccal swab samples including an engineered DNA polymerase, an specifically optimized reaction buffer system, and ultrapure dNTPs. A hot-start formulation of the included DNA polymerase suppresses false amplification. Only target specific primers and samples need to be added.

Description

DNA isolation is not needed anymore - Direct Oral Swab 2x PCR Master Mix allows direct PCR, as well as PCR based genotyping or pathogen detection directly from saliva or buccal swab samples.
The included DNA polymerase variant is engineered for superior PCR performance directly from saliva or buccal swab samples.
Direct Oral Swab 2x PCR Master Mix contains HiDi Taq DNA polymerase and thus, is also proficient in all assays in which **hi**gh single nucleotide **di**scrimination is required, for instance in allele-specific amplifications (ASA) by PCR. HiDi Taq DNA polymerase efficiently amplifies from primers that are matched at the 3'-end and discriminates primers that are mismatched.
The kit comes together with an optimized buffer system and a separate lysis buffer.
The Direct Oral Swab 2x PCR Master Mix ensures reproducible results, significantly reduces set-up times and the risk of pipetting mistakes. It can also be used for real-time cycling when using fluorescence-based hydrolysis probes.

Applications directly from saliva or buccal swab samples

- Direct PCR
- Genotyping & genomic profiling
- HLA genotyping
- SNP-detection by allele-specific amplification (ASA) / allele-specific PCR
- Pathogen detection
- End-point PCR
- Real-time PCR with fluorescence-based hydrolysis probes

Template preparation

Direct Oral Swab PCR Kit has been optimized for various sample collection kits and fresh saliva.

Sample collection kits

As amounts of stabilizing liquid vary between manufactures, it is recommended to dilute the collected sample with the provided lysis buffer to a final concentration of approximately 1-5 % saliva solution e.g. 2 µl sample (20 - 100 % saliva concentration) + 38 µl lysis buffer. As template 2.5 µl of this solution is then directly used for a 25 µl PCR reaction.

Fresh saliva

Fresh saliva should be collected in a reaction tube and centrifuged in a standard benchtop centrifuge for 20 sec at maximum speed. 2 µl of the supernatant should be mixed with 38 µl of the provided lysis buffer (5 % saliva concentration). After brief vortexing the reaction is cleared by centrifugation in a standard benchtop centrifuge for 60 sec at maximum speed. As template 2.5 µl of the supernatant is then directly used for a 25 µl PCR reaction yielding a final saliva concentration of 0.5 %.

In the case of DNA collection with buccal swabs the sample should be placed in 200 µl of the provided lysis buffer. As template 2.5 µl of the saliva solution is then directly used for a 25 µl PCR reaction.

Please note that the saliva solution of freshly collected samples without a sample collection kit can only be stored for up to 24 h at 4°C before use.

Recommendations for PCR/ Reaction Setup

PCR Mix

Component	Volume	Final concentration
Direct Oral Swab 2x PCR Master Mix	12.5 µl	1x
Primer forward (10 µM)*	0.5 µl	0.2 µM (0.05-1 µM)
Primer reverse (10 µM)*	0.5 µl	0.2 µM (0.05-1 µM)
Template/Sample extract	2.5 µl	0.1 -0.5 %
Nuclease-free water	up to 25 µl total vol.	

* Primers should ideally have a GC content of 40-60%

Typical 3-step PCR protocol

Initial denaturation	95°C	3 min	
Denaturation	95°C	10 sec	30-45 cycles
Annealing*	54-72°C	15 sec	
Extension	72°C	30 sec/ 250 bp	
Hold	<10°C		

* Typically, the annealing temperature is about 3-5°C below the calculated melting temperature of the primers used.
All times may vary depending on the PCR equipment and primer / template composition.

Recommendations for sample handling

- Keep all components on ice.
- Spin down and mix all solutions carefully before use.
- Fresh saliva samples can be stored up to 24 h at 4°C.
- Primers should ideally have a GC content of 40-60%.
- We recommend designing primers with a short amplicon length (about 60-200 bp) for best results, but also longer amplicon lengths are possible. The addition of additional magnesium chloride (+ 0.5 - 1.5 mM in the final reaction mix) might be needed in case of longer amplicons >500 bp.

Storage

This product is shipped on cool packs. Please store the product upon arrival at -20°C. Minimize the number of freeze-thaw cycles by storing in aliquots. For a day-to-day use, we recommend keeping an aliquot at 4°C.

Quality Control Assays

PCR activity: Direct Oral Swab 2x PCR Master Mix was tested for successful allele-specific PCR performance detecting a genomic SNP (rs72921001) in genomic DNA from human saliva samples. PCR products were subsequently analysed on a 2.5% agarose gel.
DNA polymerase activity: DNA polymerase activity has been monitored and adjusted to a specific DNA polymerase activity using an artificial DNA template and a DNA primer.
Enzyme-concentration has been determined by protein-specific staining. Please inquire more information at info@mypols.de for the lot-specific concentration.
No contamination has been detected in standard test reactions.

Safety

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend the use of gloves, lab coats and eye protection when working with these or any other chemical reagents. myPOLS Biotec takes no liability for damage resulting from handling or contact with this product. Further information can be found in the REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL.

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The product is for research use only and may be used for in-vitro experiments only.

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