Quality Control Assays
PCR activity: Allstar directPCR 2x Master Mix was tested for successful PCR performance. A 297 bp fragment (chloroplast) was amplified from a plant leave and analyzed by agarose gel electrophoresis.
DNA polymerase activity: Allstar 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.

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.

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.

References

1. Highly Conserved Non-Coding Sequences Are Associated with Vertebrate Development. PLoS. Biol. 2005; 3: e7. A. Woolfe, M. Goodson, D. K. Goode, P. Snell, G. K. McEwen, T. Vavouri, S. F. Smith, P. North, H. Callaway, K. Kelly, K. Walter, I. Abnizova, W. Gilks, Y. J. K. Edwards, J. E. Cooke, and G. Elgar
2. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Mol. Ecol. 1995; 4: 129–131. B. Demesure, N. Sodzi, and R. J. Petit

myPOLS Biotec, Universitätsstr. 10, 78457 Konstanz, Germany T +49(0)7531 884654

www.mypols.de
support@mypols.de
T +49(0)7531 884654

Allstar directPCR 2x Master Mix
♯3001
Store at -20°C

Contents
Allstar directPCR 2x Master Mix is containing all the components necessary for PCR, including an optimized reaction buffer, ultrapure dNTPs and an engineered DNA polymerase.
A specifically developed lysis buffer and control primer mixes for mammalian and plant DNA are also included in the kit.

Description
DNA isolation is not needed anymore - Allstar direct PCR Mix allows the PCR directly from crude samples such as blood, saliva or plants or after a quick lysis step.
Allstar DNA polymerase has been specifically selected to display a very high resistance against many types of common PCR inhibitors. The engineered DNA polymerase features robustness and selectivity. It comes together with an optimized buffer system and a separate lysis buffer.
The pre-ready 2x Master Mix ensures repeatable results, significantly reduces set-up times and the risk of pipetting mistakes.

Applications
- Direct PCR from plants and mammalian specimen
- Direct gene detection and quantification
- Standard PCR
- Realtime PCR
- Screening / Hight-throughput PCRs

Recommendations for PCR

PCR Mix

Component	Volume	Final concentration
Allstar directPCR 2x 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	x µl	according to reaction setup
Nuclease-free water		up to 25µl total volume

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

Typical 3-step PCR protocol

Initial denaturation	95°C	2 min	
Denaturation	95°C	15 sec	⎫
Annealing*	54–72°C	30 sec	⎬ 25-40 cycles
Extension	72°C	2 min/1000 bp	⎭
Hold	<10°C		

* Typically, the annealing temperature is about 3-5°C below the calculated melting temperature of the primers used.

Reaction setup/ template preparation

Saliva

Fresh saliva should be collected in a reaction tube and centrifuged in a standard benchtop centrifuge for 20s at maximum speed. 2-5% of the supernatant can directly be used in PCR without further treatment.
Alternatively saliva can be treated with a 5x excess of the provided lysis buffer. After vortexing the reaction is cleared by centrifugation in a standard benchtop centrifuge for 60s at maximum speed. The supernatant is directly used in PCR (2-5% of total reaction volume).

Blood

We recommend using 1-5% of blood (treated or untreated) directly in PCR with out further treatment.
Alternatively the blood sample (treated or untreated) can be diluted with a 2-5 fold excess of the provided lysis buffer and vortexed for one minute. The supernatant is directly used in PCR (1-5% of total reaction volume).
Please notice that the blood sample will quench the fluorescence of a realtime dye. Please check our recommendations under realtime PCR.

Plants

For plants we recommend, that a 1-4 mm^2 part of the leave is diluted with 50 µl of the provided lysis buffer and is incubated at 98° C for 1-5 min. The supernatant is directly used in PCR (1-5% of total reaction volume). Alternatively an approximately 1 mm^2 part of a leave can be directly used for PCR Also other parts of plants might be used as templates directly or after lysis.

Realtime PCR

Allstar directPCR 2x Master Mix can also be used for realtime PCR, when adding a suitable realtime dye. Please note that high concentrations of sample extracts or blood might quench the fluorescence signal.
For blood: Try increased concentrations of realtime dye or pipette the blood sample directly in the PCR tube and let it dry at room temperature for approx. 1/2h before you add remaining PCR components.

Control reactions for mammalian DNA and plant DNA

Primer control mixes for mammalian DNA and plant DNA are included in the kit. Both mixes are 10x ready to use (1 µM each). For both control mixes we recommend an extension time of 45 sec. For the plant control reaction we recommend an annealing temperature of 60°C for the mammalian control reaction an annealing temperature of 66°C.
The control primer mix for mammalians included in the kit amplifies a 237 bp fragment of highly conserved non-coding region upstream of the SOX21 gene. The primer mix contains degenerate primers and can be used with wide range of vertebrate species. Primer sequences are (also see reference 1):

Primer Mammalian 1 (24 nt) : 5'-AGCCCTTGGGGASTTGAATTGCTG -3'
Primer Mammalian 2 (27 nt): 5'-GCACTCCAGAGGACAGCRGTGTCAATA -3'

The plant specific control primer mix included in the kit amplifies a 297 bp fragment of a highly conserved region of chloroplast DNA. The primer mix can be used with a large number of plant species. Primer sequences are (also see reference 2):

Primer Plant 1 (20 nt) : 5'-AGTTCGAGCCTGATTATCCC -3'
Primer Plant 2 (20 nt) : 5'-GCATGCCGCCAGCGTTCATC -3'

Recommendations for sample handling

- Keep all components on ice.
- Spin down and mix all solutions carefully before use.
- Primers should ideally have a GC content of 40-60%.
- 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.