

ConviFlex™ DNAmP Mix

Universal PCR master mix for Conventional PCR and qPCR

PRODUCT INFORMATION

INDICATION

ConviFlex™ DNAmP Mix is a ready-to-use PCR system, designed for a broad range of applications. ConviFlex™ DNAmP Mix is compatible with both conventional PCR and qPCR (both SYBR® Green and TaqMan®-based techniques), and is suitable for all standard PCR applications. These include also PCR assays with complex genomic or cDNA templates, low copy number targets, high specificity PCR, T/A cloning, and multiplex PCR.

PRINCIPLE OF THE METHOD

ConviFlex™ DNAmP Mix is a lyophilized, universal master mix, containing hot-start Taq polymerase and dNTPs. After reconstitution, ConviFlex™ DNAmP Mix provides already all core components required for high yield PCR, except user-specific DNA template and primers.

ConviFlex™ DNAmP Mix was optimized for efficient, flexible, and reproducible PCR. With ConviFlex™ DNAmP Mix, the entire PCR reaction can be set up in few pipetting steps and with minimal hands-on time.

Due to the hot-start functionality of the Taq polymerase, the formation of unspecific PCR products and primer dimers is strongly suppressed, resulting in higher PCR specificity, increased sensitivity and greater yields.

Hot-start Taq polymerase can amplify templates up to 5 kb in length, at a detection sensitivity of ≥ 6 DNA molecules, and with a PCR extension rate of 1 kb / 1 min. ConviFlex™ DNAmP Mix can be easily adapted to most PCR protocols, programs, and cyclers types.

REAGENTS

This product contains reagents and components for 25, 100, or 250 PCR reactions. The expiry date of the unopened package is marked on the package label. Lyophilized ConviFlex™ DNAmP Mix and Rehydration Buffer should be stored at +2 - +8 °C. After rehydration, ConviFlex™ DNAmP Mix should be stored at ≤ -18 °C.

Component	Quantity			Cap color
	25 Reactions Cat. No. 191-0025	100 Reactions Cat. No. 191-0100	250 Reactions Cat. No. 191-0250	
ConviFlex™ DNAmP Mix (contains hot-start Taq polymerase and dNTPs)	1 vial, lyophilized	4 vials, lyophilized (for 25 reactions, each)	10 vials, lyophilized (for 25 reactions, each)	red
2 × Rehydration Buffer	1 vial, 1.5 ml	2 vials, 1.5 ml	4 vials, 1.5 ml	blue
MgCl ₂ (100 mM)	1 vial, 1 ml	1 vial, 1 ml	1 vial, 1 ml	violet

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The kit contains some of the components required for PCR amplification. Additional consumables and equipment are supplied by the user:

- qPCR or PCR thermocycler and microcentrifuge
- Suitable PCR reaction tubes (DNA-, RNA- and DNase- and RNase-free)
- Pipettes with corresponding filter tips (DNA-, RNA- and DNase- and RNase-free)
- User-specific DNA extracts or samples, primers and probes (only for qPCR)
- PCR grade water
- Optional for sample preparation: DNA extraction kit suitable for the collected samples

PRECAUTIONS

ConviFlex™ DNAmix is for research use only. Not for use in diagnostic procedures. ConviFlex™ DNAmix should be used by trained laboratory staff only. All samples should be handled with all due care and attention. Always wear a suitable lab coat and disposable gloves. This kit does not contain hazardous substances. Remnants can be discarded according to local regulations.

SPECIMEN

ConviFlex™ DNAmix can be used with several types of target DNA, extracted from diverse starting materials (see Related Products and our website for details and ordering information about our extraction systems).

ConviFlex™ DNAmix is also suitable for direct PCR amplification using as template cell culture supernatants or bacterial suspensions, without prior DNA extraction. This quick and convenient protocol is particularly suitable for detection of specific target DNA (e.g. pathogens). Notably, however, for complex sample matrices or matrices with high protein content (e.g. > 10 mg/ml or high serum concentrations), DNA extraction and an additional proteinase K treatment may be required prior to DNA isolation, in order to prevent PCR inhibition.

PROCEDURE - STEP BY STEP

This PCR protocol is a simple guideline to use the product. Optimal experimental conditions, (e.g. incubation steps, primers and targets concentrations) should be adjusted to the specific requirements of the assay of interest.

1. PCR reaction setup

This PCR system is intended for a 20 µl PCR reaction volume. The PCR master mix (e.g. including primers) should be prepared accordingly, by adding all necessary components to a suitable final volume and leaving an adequate volume for the sample (see table below for reaction mix recommendations).

Reaction Mix	for 1 reaction
ConviFlex™ DNAmix	10 µl
Primer mix or Primer/Probe Mix	x µl
PCR grade water (up to 15 µl)	x µl
Sample / Template / Extract	5 µl
Final Volume	20 µl

Before starting, thaw any additional component required for the assay (e.g. primers, probes, templates etc).

1. Spin down the lyophilized ConviFlex™ DNAmix (red cap) for 5 sec at maximum speed.
2. Add 260 µl of 2 × Rehydration Buffer (blue cap).
3. Incubate the rehydrated ConviFlex™ DNAmix at room temperature for 5 min, vortex briefly and spin down for 5 sec.

4. Transfer the required amount of reconstituted ConviFlex™ DNAmix into a new DNA- and DNase-free 1.5 ml tube. The required amount is calculated based on the number of reactions to be processed for each primer mix (see table above as an example guideline for such calculation).

5. Add 0.1 - 0.5 μM forward primer and 0.1 - 0.5 μM reverse primer⁽¹⁾. For TaqMan[®]-based qPCR, add 0.1 - 0.5 μM probe. For dye-based assays, refer to the concentrations recommended by the manufacturer. ⁽²⁾
6. Add PCR grade water to a final volume of 15 μl per reaction.
Mix by tapping carefully against the tube or pipetting up and down 4-5 times.
7. Aliquot 15 μl of PCR master mix to each PCR reaction tube.
8. Negative controls: add 5 μl PCR grade water or elution buffer (if DNA extraction was performed).
9. Samples: add 5 μl DNA extract / template DNA⁽³⁾.
10. Positive Control: add 5 μl template DNA, which is specifically amplified with the used primer set.
11. Close PCR tubes tightly, spin down briefly and perform PCR.

2. Standard PCR program

Commonly used PCR cycling parameters are reported in the table below. Please apply the optimal PCR program for your desired amplification. ConviFlex™ DNAmix can be successfully applied for a broad range of PCR-based assays (e.g. touchdown PCR, gradient PCR, long range PCR).

Step	Temperature [° C]	Time	Number of cycles
Initial denaturation	94	2 - 5 min	1
Denaturation	94	10 - 30 sec ⁽⁴⁾	25 - 40 ⁽⁶⁾
Annealing	T _m - 5 ⁽⁵⁾	15 - 30 sec	
Extension	72	60 sec / 1 kb of target	
Final extension ⁽⁷⁾	72	5 - 15 min	1
Hold ⁽⁷⁾	+2 - +8	indefinitely	-

(1) Primer concentrations can be increased up to 1 μM when degenerate primers are used or in longer PCR programs.

(2) Optional: if necessary, add MgCl₂ after this step to optimize your assay. The final MgCl₂ concentration obtained in the 20 μl PCR reaction by following the protocol above is 2.4 mM. We recommend a final MgCl₂ concentration of 1.5 - 6 mM.

(3) For conventional PCR, a template DNA concentration of 0.01 - 1 ng / reaction for plasmid or phage DNA, and 10 - 500 ng / reaction for genomic DNA are recommended. For qPCR, we recommend template DNA amounts of 0.01 - 1 ng / reaction for plasmid DNA and 0.1 - 500 ng / reaction for genomic DNA.

(4) In case of GC-rich DNA templates, DNA denaturation can be prolonged to 3 - 4 minutes.

(5) Primers should have the same melting temperature (T_m) when used in the same assay.

(6) For conventional PCR, we recommend 25 -35 cycles for optimal amplification results with high-copy template DNA and up to 40 cycles for detection of low-copy targets (< 10 copies of template / reaction). For qPCR, we recommend up to 40 cycles to ensure amplification of low- to single copy targets.

(7) Only for conventional PCR.

PRODUCT CHARACTERISTICS

1. Stress- and storage-testing

The lyophilized ConviFlex™ DNAmix mix remains functional after storage at temperatures up to 60 °C for 7 days.

2. In-Use-stability testing

The rehydrated ConviFlex™ DNAmix is stable even after several freeze/thaw cycles and in comparison to a freshly reconstituted mix. No performance loss has been observed.

A detailed Technical Note can be downloaded from our website: <http://www.minerva-biolabs.com>.

QUALITY CONTROL

Our quality control includes:

- Functional assays showing PCR amplification of different DNA templates.
- PCR amplification tests without templates as negative controls.
- 98 % protein homogeneity of Taq polymerase in 10 % SDS-PAGE.
- No contaminating endonuclease or exonuclease activity detected.
- Hot-start efficiency test of Taq polymerase showing effective inhibition by anti-Taq antibodies.
- Efficient 5' - 3' exonuclease activity.

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minervabiolabs.com / www.minervabiolabs.us).

APPENDIX

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising from the use, the results of use, or the inability to use this product.

Trademarks

ConviFlex, SwabUp, and ExtractNow™ are trademarks of Minerva Biolabs GmbH, Germany. TaqMan is a registered trademark of Roche Molecular Systems, Inc, and SYBR is a registered trademark of Molecular Probes, Inc..

RELATED PRODUCTS

192-0025/-0100/-0250	ConviFlex™ RT-Taq Mix (25 reactions / vial)	25/100/250 Reactions
601-1010/-1050	ExtractNow™ DNA Mini Kit	10/50 Extractions
602-1010/-1050	ExtractNow™ Blood DNA Mini Kit	10/50 Extractions
603-1010/-1050	ExtractNow™ RNA Mini Kit	10/50 Extractions
604-1010/-1050	ExtractNow™ CleanUp Kit	10/50 Extractions
605-1010/-1050	ExtractNow™ Plasmid Mini Kit	10/50 Extractions
606-1010/-1050	ExtractNow™ Virus DNA/RNA Kit	10/50 Extractions
607-1010/-1050	ExtractNow™ Vegan Control	10/50 Extractions
608-1010/-1050	ExtractNow™ Meat ID	10/50 Extractions
609-1010/-1050	ExtractNow™ Food Control	10/50 Extractions
611-1250	ExtractNow™ Virus RNA Kit	250 Extractions
611-2250	ExtractNow™ Virus RNA Swab Kit	250 Extractions
181-0010/-0050	SwabUp™ Lab Monitoring Kit	10/50 Extractions
56-0002	Proteinase K	50 Extractions

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