

ConviFlex™ RT-Taq Mix

Universal One-Step RT-PCR Mix for RT-qPCR

Indication

ConviFlex™ RT-Taq Mix is a lyophilized universal RT-PCR master mix, designed for the qualitative detection and analysis of RNA molecules, particularly suitable for detection of RNA-based viruses and functionality.

Principle of the Method

ConviFlex™ RT-Taq Mix is a ready-to-use one-step RT-PCR kit containing a MuLV reverse transcriptase, a hot start Taq polymerase, and dNTPs in a lyophilized master mix. After reconstitution with the supplied 2x rehydration buffer, ConviFlex™ RT-Taq Mix already consists of all core components required for RT-PCR amplification in one step, except for the RNA template and primers. This allows to greatly reduce the number of pipetting steps required to set up the RT-PCR reaction, the hands-on time, and the risk of cross-contaminations.

The Taq polymerase included in ConviFlex™ RT-PCR Mix has a hot start functionality. Its activity is blocked through anti-Taq DNA polymerase antibodies during the PCR set up even at ambient temperatures. This inhibition is completely reversed during the denaturation when the temperature increases above 70 °C, resulting in the activation of the Taq polymerase. Thus, the formation of unspecific PCR products and primer-dimers is strongly suppressed, resulting in PCR reactions with higher specificity, increased sensitivity, and greater yields. The reverse transcriptase included in the mix is a genetically modified from murine leukemia virus (M-MuLV).

ConviFlex™ RT-PCR Mix can easily be adapted to most RT-PCR protocols, programs and cycler types. For this reason, the RT-PCR set up and protocols in this document are only recommendations meant to assist you in the use of the RT-PCR system. Optimal volumes, concentrations, temperatures and incubation time may greatly vary for your specific application and should be adjusted to the requirements of your own assay.

Reagents

Each kit contains reagents for 25, 100, and 250 reactions (25 reactions / vial). The expiry date of the unopened package is marked on the package label. Lyophilized ConviFlex™ RT-Taq Mix should be stored at +2 - +8 °C, and after rehydration at ≤ -18 °C.

Component	Quantity			Cap color
	Cat. No. 192-0025	Cat. No. 192-0100	Cat. No. 192-0250	
Rehydration Buffer	1 vial, 1.5 ml	1 vial, 1.5 ml	2 vials, 1.5 ml	blue
ConviFlex™ RT-Taq Mix	1 vial, lyophilized	4 vials, lyophilized	10 vials, lyophilized	red

User-supplied consumables and equipment

The kit contains components for RT-PCR amplification. Additional consumables and equipment are supplied by the user:

- Target gene-specific primers and user-specific samples. Optional: Probes for qPCR
- qPCR thermocycler
- DNase- and RNase-free qPCR reaction tubes
- Pipettes with corresponding filter tips (DNase- and RNase-free)
- Microcentrifuge

Specimen

ConviFlex™ RT-Taq Mix can be used for retrotranscription and PCR amplification of eukaryotic, prokaryotic, viral RNA, and *in vitro* transcribed RNA. RNA may be extracted from diverse starting materials like tissue, swabs and biopsies, mammalian cells and bacteria with a commercially-available RNA extraction kit (see ExtractNow™ RNA Mini Kit Cat. No. 603-1050 or ExtractNow™ Virus RNA Kit Cat. No. 616-1010/616-1050) or a well-established extraction method. The RNA preparation must be free of RT-PCR inhibitors such as organic solvents, etc. The degradation of the extracted RNA greatly limits the performance of RT-PCR reactions. Minimizing the number of freeze-thaw cycles of RNA samples or RNase contaminations helps preventing RNA degradation. DNA contamination can also reduce the RT-PCR yield.

Precautions

ConviFlex™ RT-Taq Mix should be used by trained laboratory staff only. All samples should be handled with all due care and attention, according to the specimen type. Always wear a suitable lab coat and disposable gloves. This kit does not contain hazardous substances. Remnants can be discarded according to local regulations.

Additional Notes

- These instructions must be understood to successfully use the ConviFlex™ RT-Taq Mix. The reagents supplied should not be mixed with reagents from different lots but used as an integral unit. The reagents of the kit must not be used beyond shelf life.
- Follow the exact protocol of preparing ConviFlex™ RT-Taq Mix. Deviations may affect the test method and results.
- We recommend including control samples to monitor the reliability of your results and for troubleshooting. Set up at least one positive and one negative control sample (and/or non-template control, NTC) in each PCR. Use the elution buffer in case of extracted RNA. The control samples must be processed in the same manner as the test samples. You may want to include other lab specific control samples such as high, median and low RNA levels.
- To avoid DNA and unspecific RNA cross-contaminations during the procedure, we recommend performing the RT-PCR under RNA- and DNA-free conditions.
- RNA template quality: RNA degradation greatly limits the RT-PCR performance. Minimizing the number of freeze-thaw cycles of RNA samples or RNase contaminations helps preventing RNA degradation. DNA contamination can also greatly reduce the RT-PCR yield. Please follow the recommendations for your specific RNA sample preparation to prevent degradation and ensure high sample quality.
- RNA template concentration: The optimal amount of input RNA in a RT-PCR reaction depends on several factors, including the quality or origin of the extracted RNA, or the relative abundance of your gene of interest. For low-copy genes, higher amounts of template RNA should be used.
- Primer concentration: The recommended concentration range for primers and probes (when applicable) is 0.1 - 0.5 μM . Excessive primer concentrations may increase primer mismatches formation and generation of non-specific RT-PCR products. Nevertheless, in case of longer RT-PCR programs or the use of degenerate primers, higher primer concentrations of up to 1 μM are recommended. Primers should have the same melting temperature when used in the same RT-PCR assay.
- Retrotranscription: Here we recommend a temperature of 50 °C for the RT step. However, this temperature or the step duration may be optimized to enhance the performance of the reaction.

Procedure - Step-by-Step

1. Reagent preparation

1.1.			Spin down for 5 sec at maximum speed.
1.2.	ConviFlex™ RT-Taq Mix	red cap	Add 260 μ l 2 \times Rehydration Buffer (blue cap).
1.3.			Incubate the mix at room temperature for 5 min, vortex briefly and spin down for 5 sec.

2. RT-PCR reaction mix preparation

	Recommended pipetting scheme:		
		For 1 reaction	For 25 reactions
	ConviFlex™ RT-Taq Mix	10 μ l	250 μ l
2.1.	Forward and reverse primers (μ M)	0.1 - 0.5 μ M	
	Probes (μ M)	0.1 - 0.5 μ M	
	PCR Grade Water	to 15 μ l	to 375 μ l
2.2.	Mix by tapping carefully against the tube or pipetting up and down 4 - 5 times.		
2.3.	Aliquot 15 μ l PCR master mix to each PCR reaction tube.		
2.4.	Add 5 μ l of RNA template. or Add 5 μ l elution buffer from RNA extraction kit as Negative Control (extraction). or Add 5 μ l PCR grade water as No-Template Control (PCR). or Add 5 μ l Positive Control.		
2.5.	Close PCR tubes tightly and spin down briefly.		
2.6.	Place PCR tubes in the cyclor and close the lid tightly.		
2.7.	Program the cyclor or load a stored cyclor program, as for example ^a : 1 cycle 50 °C for 20 min 1 cycle 95 °C for 10 min 45 cycles 95 °C for 15 sec 60 °C for 1 min Hold between +4 °C and +10 °C		
2.8.	Start the program.		

^aThe following RT-PCR program are examples based on our own experience, which are meant to assist you with the use of the ConviFlex™ RT-Taq Mix. You should always adjust temperatures and incubation time to the requirements of your own assay.

Catalog Number

192-0025	25 Reactions
192-0100	100 Reactions (25 reactions / vial)
192-0250	250 Reactions (25 reactions / vial)

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 is a trademark of Minerva Biolabs GmbH, Germany.

Related Products

191-0025/-0100/-0250	ConviFlex™ DNAm Mix (25 reactions / vial)	25/100/250 Reactions
616-1010/616-1050	ExtractNow™ Virus RNA Kit	10/50 Extractions

Germany:

Minerva Biolabs GmbH

Schkopauer Ring 13 | 12681 Berlin | Germany
Tel.: +49-30-2000437-0 | Fax: +49-30-2000437-9
E-mail: order@minerva-biolabs.com
Internet: www.minerva-biolabs.com

USA / Canada:

Minerva Biolabs Inc.

1 Jill Ct., Building 16, Unit 10
Hillsborough | NJ 08844 | USA
Phone: 1-908-524-4661
order@minervabiolabs.us | www.minervabiolabs.us