3B BlackBio Biotech India
A joint venture of Kilpest India Limited, 2B BlackBio, S.L. and Biotools B&M Labs, S.A. Madrid, Spain

7-C, Industrial Area, Govindpura
Bhopal-462 023

Tel. +91-755-4077847
Fax +91-755-4282659
E-mail: info@3bblackbio.com, orders@3bblackbio.com
Website: www.3bblackbio.com

3B RetroTools® Two Step KIT

with DNA-Amplification Buffer $MgCl_2$ FREE
including a recombinant Tth DNA Polymerase thermostable

STORE AT -20ºC

The RetroTools® Two Step Kit allows the amplification of a specific RNA sequence using a single enzyme, *Tth* DNA Polymerase, in a two step process in one tube. The *Tth* DNA Polymerase catalyses in the presence of Mn^{2+} and RNA template the polymerization of nucleotides in to cDNA, whereas in the presence of Mg^{2+} the enzyme catalyses the polymerisation of dNTPs into duplex DNA. The thermostability of the *Tth* DNA Polymerase confers higher specifity to the kit because the transcription reaction can be performed at elevated temperatures. Furthermore, secondary ARN structures can be resolved employing higher temperatures.

RT-PCR PROTOCOL USING SPECIFIC *PRIMERS*

1st-STEP: Reverse transcription (RT).

Add the following reagents to a sterile tube:

5X RT Buffer	2 µl
5mM$MnCl_2$ Solution	2 µl
dNTP Mix,10mM each	0,25 µl
RNA Template	10 pg to 1 µg
Downstream primer	20 pmoles
RetroTools® Polymerase	1,25 µl
Double distilled sterile water	To a final volume of 10 µl

Incubate the reaction mixture at 60ºC-70ºC for 15 to 60 minutes. The incubation time will depend on the length of the desired fragments and must be optimised accordingly. The pre-annealing (see Note 1) and annealing temperatures used for the reverse transcription reaction should also be optimised for each *downstream primer*.

NOTE 1: *In case the downstream primer has a Tm lower than 50ºC,* ***it is recommended including a preannealing step of 3 minutes at 45ºC-50ºC*** *in order to increase the efficiency of the transcription reaction, and consequently the amplification yield.*

NOTE 2: *Once the RT has finished, keep the vials on ice, and* ***immediately*** *proceed to the next step, or keep them at -20ºC. If the vials are frozen add 0.3 µl fresh RetroTools® polymerase to the amplification reaction, as freezing inactivates the enzyme in the mixture*.

NOTE 3: *For detection of low-copy messengers, a cyclic reverse transcription protocol is highly recommended: 94ºC for 1 min (initial denaturing step) + 4 cycles (48ºC for 5 min, 60ºC for 15 min).*

2nd-STEP: Amplification Reaction.

Once the reverse transcription has been performed, the reaction volume for each tube should be made up 50 µl by adding the following reagents:

	8 µl
5X DNA-Amplification Buffer $MgCl_2$ FREE	
"Upstream" primer	20 pmoles
50 mM $MgCl_2$ Solution	Minimum 2 µl (2 mM final concentration)
Doubledistilledsterile water	to 40 µl

Recommended conditions for the amplification:

94ºC	for 2 minutes	Initial denaturing
94ºC	for 15 seconds	x 30 to 45 cycles
55-65ºC	for 30 seconds	
72ºC	1 minute per Kb	
72ºC	for 3 minutes	Final extension
4ºC		

The annealing temperature depends on the Tm of the primer pair used. The products obtained can be analysed by electrophoresis in agarose or polyacrylamide gels.

RT-PCR PROTOCOL USING NON-SPECIFIC *PRIMERS FOR THE RT*

1st-STEP: Reverse transcription (RT).

Add the following reagents to a sterile tube:

5X RT Buffer	6 µl
5mM$MnCl_2$ Solution	6 µl
dNTPMix,10mM each	0,25-0,5 µl
Non-specific primers	5-10 pmoles
RNA Template	10 pg to 1 µg
RetroTools® polymerase	1,5 µl
Bidistilledsterile water	To a final volume of 30 µl

Incubate the reaction mix at 42ºC for 5 minutes. Increase the temperature slowly to 60-65ºC (the use of the **Ramp** option of thermal cyclers is strongly recommended) and incubate the reaction mixture for 30-60 minutes. The incubation time will depend on the length of the desired fragments, and must thus be accordingly optimised.

Following the reverse transcription reaction, proceed immediately to the amplification step. As a general rule, use 10 µl of the former reaction as template, and store the remaining at -20ºC. When detection of rare messengers is our aim, the template volume for the amplification reaction can be increased up to 30 µl.

> ***NOTE:*** *For using the stored template, it is necessary to add 0.3 µl of fresh RetroTools® polymerase, as freezing inactivates the enzyme.*

2nd-STEP: Amplification Reaction.

Once reverse transcription has been performed, add the following reagents to 10 µl of the RT reaction (see above), bringing the final volume up to 50 µl.

5X DNA-Amplification Buffer $MgCl_2$ FREE	8 µl
50 mM $MgCl_2$ Solution	Minimum 2 µl (2 mM final concentration)
Downstream primer	5-10 pmoles
Upstream primer	5-10 pmoles
Bidistilled sterile water	to 40 µl

Recommended reaction conditions for the amplification:

94ºC	2 minutes	Initial denaturing
94ºC	15 seconds	x 30 to 45 cycles
55-65ºC	30 seconds	
72ºC	1 minute per Kb	

72ºC	3 minutes	Final extension
4ºC		

The annealing temperature depends on the Tm of the pair of specific primers used. The products obtained can be analysed by electrophoresis in agarose or polyacrylamide gels.

Ordering Information

PRODUCT	FORMAT	REF.
RetroTools® Two Step KIT with DNA-Amplification Buffer with $MgCl_2$	100 rxn	3B124
RetroTools® Two Step KIT with DNA-Amplification Buffer $MgCl_2$ FREE	500 rxn	3B125