



BIOTOOLS

BIOTOOLS B&M LABS.S.A.

SCRIPTOOLS OneStep Kit
(includes a thermostable retrotranscriptase)

**One step system for
efficient and robust RT-PCR**

Cat. No. 10.071/2/3

Store kit upon arrival at -20°C .

Please check integrity of the Kit and the reagents before use.

1. DESCRIPTION

SCRIPTOOLS OneStep Kit is a one step system for an efficient and specific reverse transcription (RT) of RNA molecules and amplification (PCR) of the formed cDNA.

The components of the Kit have been optimized for convenient and reliable RT-PCR. For these purpose two high-performance enzymes, a thermostable reverse transcriptase working at a temperature range of $37-77^{\circ}\text{C}$, and a DNA polymerase with proofreading activity carry out the reaction. Both cDNA synthesis and PCR are performed sequentially in a single tube due to a single buffer system formulated to ensure specific primer annealing over a wide range of temperatures. The reverse transcriptase includes in the kit resolves difficult RNA templates such as secondary ARN structures or sequences rich in G+C content.

For its use simply add to a vial containing SCRIPTOOLS Buffer, MgCl_2 and SCRIPTOOLS Enzyme Mix the desired primers and RNA template. One step format lowers contamination risks and reduces variability providing high yield, sensitivity and efficiency. We recommend the use of gene-specific primers designed with a T_m high enough to perform the RT at $42-65^{\circ}\text{C}$.

NOTE: *The Kit is **not** recommended for certain experiments dealing with sequences homologous to those found in *E. coli*. **The amplification product obtained with the Kit is blunt-end.***

2. CONTENTS OF THE KIT AND STORAGE

The Kit contains reagents for performance of 25 rxn of 50 μ l (Cat. No. 10.071), 50 rxn of 50 μ l (Cat. No. 10.072) or 100 rxn of 50 μ l (Cat. No. 10.073). The reagents are in liquid format. **Store Kit at -20°C.**

- **SCRIPTOOLS BUFFER:** a single buffer mixture including dNTPs at the appropriate concentration. It has been formulated to optimized reverse transcription and PCR amplification. **Store vial at -20°C.**
- **MgCl₂ solution** (50 mM): initial testing at several MgCl₂ concentration is advisable. **Mix well before use. Store at -20°C.**

- **SCRIPTOOLS ENZYME MIX:** Optimized mixture of a thermostable reverse transcriptase and Biotools *Pfu* DNA polymerase with proofreading activity. **Store at -20°C.**

Cat. No.	ScripTools Buffer	ScripTools Enzyme Mix	MgCl ₂ solution
10.071 (25 rxns)	180 µl	100 µl	1.8 ml
10.072 (50 rxns)	2X 180 µl	2X 100 µl	1.8 ml
10.073 (100 rxns)	4X 180 µl	4X 100 µl	1.8 ml

NOTE: *Do not freeze/thaw the reagents repeatedly. In case of frequent use, we recommend the aliquoting of the vial contents.*

3. MATERIALS TO BE SUPPLIED FOR THE USER

- Reaction vials
- pipettes
- Upstream and downstream primers
- RNase-free water
- RNA

4. INSTRUCTIONS FOR USE

Prepare the master mix in the reagent preparation area. Thaw and handle Kit reagents and primers on ice. Keep reaction vials refrigerated until their introduction in the thermal cyclor. Use of

reaction mixes and vials in non-refrigerated conditions may cause a drastic decrease in sensitivity and quality of the obtained results.

1.- Final reaction volume is 50 μ l (Master Mix + Template RNA). **Prepare the Master Mix** following the Table 1 according to the number of reactions to be performed. For each round of analysis include one negative control NTC (control without RNA). To ensure sufficient volume for all desired reactions include additional reactions in the calculations. Mix gently and thoroughly the Master Mix.

Table 1. Reaction Mixture.

COMPONENT		Volume 1 Reaction	Final Concentration
MASTER MIX	ScripTools Buffer	6 µl	1X
	MgCl ₂ (50 mM)*	0.75 µl	0.75 mM
	ScripTools Enzyme Mix	3.50 µl	--
	RNase-free water ⁺	variable	--
	Downstream primer ⁺	variable	0.5-1 µM
	Upstream primer ⁺	variable	0.5-1 µM
TEMPLATE RNA	PURIFIED RNA	variable	<1 µg
TOTAL REACTION VOLUME		50 µl	

**For each experiment the optimal MgCl₂ concentration must be determined*

**Not supplied with the Kit*

2.- Dispense the appropriate volume of **Master Mix** in each reaction vial and store vials on ice. The master mix contains the reagents necessary for the RT-PCR except the template RNA

Efficiency of the reaction depends on the quality of RNA template therefore intact RNA is essential for good results. Proceed to an area separate from that used for DNA preparations. Thaw and handle RNA on ice. RT-PCR must start in the next 2 minutes after adding RNA template.

3.- Add **RNA template** (max conc 1 µg) to each reaction vial containing the master mix. If necessary complete up to 50 µl final reaction volume with

RNase-free water. The quantity of RNA depends on the characteristics of the assay.

4.- Negative Controls without RNA template (NTC) should be prepared with **RNase-free water** instead. This control indicates the existence of DNA/RNA contamination in any reagent. Keep reaction vials on ice.

5.- Close the reaction vials and gently mix them without creating bubbles but do not vortex them. For thermal cycler without heated lid overlay a mineral oil layer.

Proceed to work in the amplification area.

6.- Program the thermal cycler according to the instructions below. Place the reaction vials in the thermal cycler once the equipment has reached the temperature for reverse transcription and run the program. Store the remainder of the Kit reagents at the appropriate temperature.

Table 2. Guide to Thermal Cycling Program.

<i>STE</i>	<i>TEMP</i>	<i>TIME</i>	<i>COMMENTS</i>
RT	42-65°C	30 min	<i>First strand cDNA synthesis</i>
1 Cycle	94°C	2 min ⁺	<i>Transcriptase is inactivated and cDNA is denatured</i>
PCR*	<i>Second strand cDNA synthesis and PCR</i>		
40-60 Cycles	94°C	20 sec	<i>Denaturation</i>
	T _m -5°C	30-60 sec	<i>Annealing (approximately 5°C below the calculated T_m of the primers)</i>
1 Cycle	72°C	Size dependent	<i>Extension depends on the size of the amplicon allowing for: 100-250 bp/45 sec Amplicon > 250 bp 1 min/500 bp</i>
	72°C	5 min	<i>Final extension</i>

⁺The time for inactivation of the reverse transcriptase must be kept to 2 min.

*Optimize the time, the temperature and the number of cycles of the PCR. The annealing time varies from 30-60 sec. For the annealing temperature start by performing the amplification 5°C below the T_m of the primers and increase the

temperature in 1°C increments. The extension time varies with the size of the amplification product approximately 1 min/500 bp, for small amplicons <250 bp allows 45 sec. Once all the parameters have been optimized determine empirically the number of cycles

7.- After amplification samples can be stored overnight at 2-8°C, or at -20°C for longer-term storage.

NOTE: *The obtained amplification product is blunt-end*

5. TROUBLESHOOTING

Problem	Cause	Recommendation
No amplification product or low yield	Missing reagent or pipetting error	Check reaction components and repeat the assay.
	Degraded primer or RNA	Verify the quality of the RNA and primers. Ensure that all material used are RNase-free.
	Incorrect or poor primer design	Check design of the primers, if necessary redesign them (verify length, T_m value, complementarity to the appropriate strand, self complementarity or between primers). A 0.8 μM primer concentration is recommended. Repeat the RT-PCR using different concentrations of the primers (0.5-1 μM in 0.1 increments).
	Thermal cycler program incorrect	Certain positions in the thermal cycler do not reach the expected temperature. Determine the problematic positions. Check temperature, time and number of cycles.
	No first strand product	Verify the quality of RNA, RT-primer specificity and RT program.

	RT reaction not optimal	<p>Perform the RT according to the T_m of the RT-primer. The included reverse transcriptase exhibits a robust cDNA synthesis from 37-77°C For RNA templates prone to secondary structures increase the temperature up to 77°C.</p> <p>Low abundance targets or long amplicons may need to increase the time of the RT step up to 60 min.</p>
Problem	Cause	Recommendation
Multiple non specific amplification products	Incorrect conditions for reverse transcription	Keep reaction tubes on ice until their introduction in the thermal cycler. Preheat the thermal cycler at the appropriate RT temperature.
Multiple non specific amplification products	Suboptimal RT-PCR conditions	Check primer design. Increase the PCR annealing temperature.
	Contamination	Presence in the sample of another target RNA/DNA. Check the quality of the employed RNA.
	Multiple targets in the sample RNA	Design new primers.

Amplification product has higher size than expected	Sample contaminated with DNA sequences related to the RNA	Digest the DNA present in the sample using DNase.
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6. WARRANTY

Products are guaranteed to conform to the quality and content indicated on each vial and external labels during their shelf life. BIOTOOLS obligation and purchaser's rights under this warranty are limited to the replacement by BIOTOOLS of any product that is shown defective in fabrication, and that must be returned to BIOTOOLS, freight prepaid, or at BIOTOOLS' option, replacement of the purchasing price.

Any complaint on damaged goods during transport must be directed to the handling or transport agent.

Manufactured by:

BIOTOOLS, Biotechnological & Medical Laboratories, S.A. has been evaluated and certified to accomplish ISO 9001:2000 requirements for the following activities: Research and development of biotechnology products and manufacture of biotechnology and in vitro products.
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