

Standard reverse transcription PCR protocols are typically time-consuming and extremely prone to contamination. This situation compromises an already highly sensitive reaction. However, with the development of thermostable reverse transcription enzymes and the advent of MBP's HotStart Storage and Reaction Tubes, RT-PCR has been made quicker, easier and virtually contamination free.

By utilizing a wax barrier as a separation medium, RT-PCR can now be completed in one tube. In doing so, the concern for sample loss and contamination involved in transferring reagents from tube to tube is eliminated. Also, because the enzyme used in this protocol is thermostable, reverse transcription time is cut in half. The specificity and sensitivity associated with RT-PCR is also heightened as the separation of reaction layers provides for a "hot start" PCR reaction.

The following is a general protocol for using HotStart tubes for RT-PCR; reactions for specific templates may need optimization. Using a HotStart 50[®] or HotStart Micro 50[®] for a total reaction volume of 50 µl, or a HotStart 100[®] or HotStart Micro 100[®] for a total reaction volume of 100 µl, pre-mix the following ingredients - or add individually - to the bottom of the tube:

Reagent	50 µl Reaction	100 µl Reaction
DEPC-treated H ₂ O	5.2 µl	10.4 µl
Template RNA	0.5 µl (125 ng)	1.0 µl (250 ng)
Reverse Transcription Buffer, 10x ¹	1.0 µl	2.0 µl
10mM MnCl ₂ Solution	1.0 µl	2.0 µl
dNTPs, 2.5mM each	0.8 µl	1.6 µl
rTth DNA Polymerase*	1.0 µl	2.0 µl
oligo dT 12-18	0.5 µl (0.25 µg)	1.0 µl (0.5 µg)
TOTAL VOLUME	10.0 µl	20.0 µl

Place in pre-heated thermal cycler at 70° C for 15 minutes, then cool to room temperature, completing the reverse transcription reaction. Then either pre-mix - or add individually - the following ingredients on top of the wax barrier:

Reagent	50 µl Reaction	100 µl Reaction
Autoclaved dH ₂ O	31 µl	62 µl
Chelating Buffer, 10x ²	4.0 µl	8.0 µl
25m M MgCl ₂	4.0 µl	8.0 µl
Primer A	0.5 µl (10 µM)	1.0 µl (10 µM)
Primer B	0.5 µl (10 µM)	1.0 µl (10 µM)
TOTAL VOLUME	40.0 µl	80.0 µl

Carry out the regular DNA amplification procedure required for the template (30-35 cycles). Due to the separation of template DNA from the primer and Mg²⁺ ion, false priming is discouraged. Remove your PCR product from the tube after amplification for analysis.

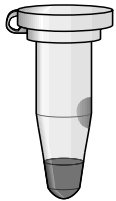
¹RT buffer: 100 mM tris-Cl pH 8.3, 900 mM KCl.

²Chelating buffer: 50% (v/v) glycerol, 100 mM tris-Cl pH 8.3, 1M KCl, 0.5%, tween 20, 7.5 mM EGTA.

RT-PCR In One Tube

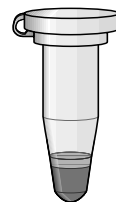
Step 1

Add Reverse Transcription Reagents



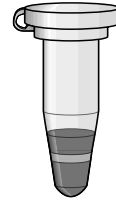
Step 2

Heat at 70° C for 15 minutes
(Then allow wax to cool)



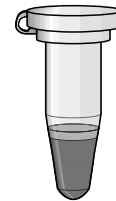
Step 3

Add PCR Reagents



Step 4

Complete a PCR cycles



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* Perkin-Elmer DNA polymerase, part No. N808-0097 (has RT activity).

** Applied Biosystems