

cDNA Synthesis Protocol

This one step protocol facilitates the synthesis of single stranded cDNA from mRNA. The strand synthesis is driven by AMV and oligo(dT) Primer.

RNA: it depends on the concentration of RNA

H₂O: it depends on the concentration of RNA

H ₂ O	?
10 X Buffer	2 µl
MgCl ₂	4 µl
RNasin	0.6 µl
dNTPs	2 µl
oligodt	1 µl
AMV	1 µl
RNA	?
	21 µl

- ✚ Incubate in 42 °C for 1 h.

cDNA Synthesis for RT-PCR Protocol

This two steps protocol facilitates the synthesis of cDNA of RNA in order to be used in the Real Time PCR.

- ✚ Add the following components to a nuclease-free microcentrifuge tube.

1µg total RNA

10pM oligo(dT)s

1µM dNTPs

20U RNase inhibitor

DNase-RNase free water

- ✚ Heat mixture to 65 °C for 5 min and cool on ice for at least 1 minute.
- ✚ As long as the above mixture is on PCR, prepare the second tube.

Buffer 5x

5mM DTT

RNase inhibitor

200U SuperScript III Reverse Transcriptase

- ✚ Transfer the mixture of the second tube to the first tube and mix gently.

- ✚ Incubate tube at 25 °C for 5 min.
- ✚ Then, incubate at 50 °C for 50 min.
- ✚ Inactivate at 70 °C for 15 min.
- ✚ Store at –80 °C.