

# Reverse Transcriptase Core kit Technical Data Sheet

## Reference: RT-RTCK-03

Products and procedures describe in this protocol are intended for research purposes only.

### Storage conditions

For long term storage the Reverse Transcriptase Core kit should be stored at -65 °C to -75 °C in a constant temperature freezer. When stored under these conditions the reagents are stable for 1 year.

For short term storage the Reverse Transcriptase Core kit can be stored at -15 °C to -25 °C for 6 months.

### Kit contents

The Reverse Transcriptase Core kit contains enough reagents for up to 300 - 10 µl reactions.

Reagent	Volume	Description
10x reaction buffer (black cap)	1.4 ml	One bottle of RT reaction buffer KCl and Tris-HCl
EuroScript reverse transcriptase (white cap)	75 µl	One tube of Moloney Murine leukemia virus reverse transcriptase, 6250 U at 50 U/µl
RNase Inhibitor (purple cap)	120 µl	One tube of RNase inhibitor 4000U at 20 U/µl
2.5 mM dNTP Mix (green cap)	1.25 ml	One tube of dATP, dCTP, dGTP and dTTP in autoclaved, deionized water titrated with NaOH to pH 7.0
25 mM MgCl <sub>2</sub> (orange cap)	1.5 ml	One tube of 25 mM MgCl <sub>2</sub>
Oligo d(T) <sub>15</sub> VN (yellow cap)	150 µl	One tube containing 50 µM oligodeoxynucleotides of sequence d(T) <sub>15</sub> VN in 10 mM Tris-HCL, pH 8.3
Random nonamers (pink cap)	150 µl	One tube containing 50 µM short oligonucleotides of random sequence (d(N) <sub>9</sub> ) in 10 mM Tris-HCL pH 8.3
RNase free water (plain cap)	1.75 ml	One tube of DEPC water

### Procedure for Two step RT qPCR reaction

1- Thaw all required reagents necessary for the RT step completely and put them on ice, except for the EuroScript, which should be kept in the freezer until required for use. Mix all reagents well by inversion and spin them down prior to pipeting.

2- Prepare the RT Reaction Mix  
(sufficient for 200 ng total RNA per 10 µl RT step)

Component	Volume (µl)	Final concentration
10x reaction buffer	1	1x
25 mM MgCl <sub>2</sub>	2	5 mM (or as required)
2.5 mM dNTP	2	500 µM each dNTP
Random nonamer*	0.5	2.5 µM
RNase Inhibitor	0.2	0.4 U/µl
EuroScript RT	0.25	1.25 U/µl
RNase free water	3.05	-
Template	1	10 pg - 200 ng Total RNA
<b>Total Mix</b>	<b>10 µl</b>	

\*Note: random nonamers, oligo d(T)<sub>15</sub>VN or sequence specific primers can be used for primers. For nonamers and oligo d(T)<sub>15</sub>VN the final concentration in the reaction mix should be 2.5 µM. For a sequence-specific reverse primer, the final concentration should be 200 nM.

2.1- To correct for dispensing losses prepare an excess of reaction mix (for example 100 reactions reaction mix for 96 reactions). Add all components together, except for the template. Mix thoroughly by inversion. Spin down.

2.2- Add the reaction mix to the reaction vial. reaction set up should be done on ice

2.3- Add the template to individual reactions, gently mix by inversion. Spin down. A negative control containing no RNA template should always be included. Optionally a no RT-control should be set up in tubes / wells, which do not contain the EuroScript RT / RNase Inhibitor.

2.4- Program the Real-Time thermocycler using the following recommended parameters:

<b>Initial step*</b>	10 min 25 °C
<b>Reverse Transcriptase step</b>	30 min 48 °C
<b>Inactivation of the RT enzyme</b>	5 min 95 °C

\* Only if random nonamers or oligo d(T)<sub>15</sub>VN are used.

**The RT Core kit can be combined with any Eurogentec qPCR kits**

3- Thaw all required reagents necessary for the PCR step completely and put them on ice. Mix all reagents well by inversion and spin them down prior to pipeting.

**4- Prepare the PCR Reaction Mix**

4.1- In case of a probe assay:

Component	Volume (µl)	Final concentration
Reaction buffer	5* 25**	1x
50 mM MgCl <sub>2</sub>	5 -	5 mM (or as required)
5 mM dNTP	2 -	200 µM each dNTP
Forward primer	5 5	-
Reverse primer	5 5	-
Probe	5 5	-
HotGoldStar	0.25 -	0.025 U / µl
RNase free water	7.75 -	-
<b>Total Mix</b>	<b>40 µl</b>	

\* If using a qPCR Core kit

\*\* If using a qPCR MasterMix

**Add 40 µl of PCR reaction Mix to 10 µl or a dilution of the 1st strand reaction mix.**

4.2- In case of a SYBR® green I assay

Component	Volume (µl)	Final concentration
Reaction buffer	5* 25**	1x
50 mM MgCl <sub>2</sub>	5 -	5 mM (or as required)
5 mM dNTP	2 -	200 µM each dNTP
Forward primer	5 5	-
Reverse primer	5 5	-
diluted SYBR green I	1.5 -	-
HotGoldStar	0.25 -	0.025 U / µl
RNase free water	7.75 -	-
<b>Total Mix</b>	<b>40 µl</b>	

\* If using a qPCR Core kit for SYBR® green I

\*\* If using a qPCR MasterMix Plus for SYBR® green I

**Add 40 µl of PCR reaction Mix to 10 µl or a dilution of the 1st strand reaction mix.**

4.3- To correct for dispensing losses prepare an excess of reaction mix (for example 100 reactions reaction mix for 96 reactions). Add all components together, except for the template. Mix thoroughly by inversion. Spin down.

4.4- Add 10 µl or a dilution of the first strand reaction mix, 5 µl of the template control (plus 5 µl of water or buffer) for your positive control and 10 µl of water or buffer for your negative control in to your PCR tubes / 96-well plate / 384-well plate.

4.5- Add 40 µl of the reaction mix to the reaction vial, close the vial and mix gently on a stirrer or spin down. Ensure that no bubbles are present in the reaction vial. Reaction set up can be done at room temperature.

4.6- Program the Real-Time thermocycler using the following recommended parameters:

UNG step	2 min. 50 °C
HotGoldStar activation/UNG inactivation	10 min. 95 °C
40 Cycles	15 sec. 95 °C 1 min. 60 °C
Hold	50 °C forever

For any further informations concerning the qPCR step, in terms of primer and probe design, primer and probe concentrations, MgCl<sub>2</sub> concentration or concerning optimization of the reaction, please refer to the instruction of the Eurogentec qPCR Core kits or qPCR MasterMixes.

**Further information available through Eurogentec web site, [www.eurogentec.com](http://www.eurogentec.com).**

- Troubleshooting Guide for qPCR and RTqPCR (under the "Technical Resources / Troubleshooting Guide" section).

- Primers and probe design (please refer to our Troubleshooting Guide).

- "Your One-stop-shop Real-Time qPCR supplier" handbook (under the "Technical Resources / Documentation" section).

- MSDSs, (under the "Technical Resources / MSDS" section)

- Certificates of Analysis (please contact us).

**For any further information required please contact our Customer Help Desk:**

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Tel : +32 4 372 76 65

*For USA:*

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