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Reverse Transcriptase Core kit Technical Data Sheet

Reference: RT-RTCK-03

Products and procedures described in this document 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 const ant 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, 3750 U at 50 U/µl
RNase Inhibitor (purple cap)	120 µl	One tube of RNase inhibitor 2400U 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 $MgCl_2$ (orange cap)	1.5 ml	One tube of 25 mM $MgCl_2$
Oligo $d(T)_{15}VN$ (yellow cap)	150 µl	One tube containing 50 µM oligodeoxynucleotides of sequence $d(T)_{15}VN$ in 10 nM Tris-HCL, pH 8.3
Random nonamers (pink cap)	150 µl	One tube containing 50 µM short oligonucleotides of random sequence $(d(N)_9)$ in 10 mM Tris-HCL pH 8.3
RNase free wate (plain cap)	1.75 ml	One tube of DEPC water

Procedure for Two step qRT-PCR 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 up to 200 ng of total RNA per 10 µl RT step)

Component	Volume (µl)	Final concentration
10x reaction buffer	1	1x
25 mM $MgCl_2$	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
Total Mix	**10 µl**	

*Note: random nonamers, oligo $d(T)_{15}VN$ or sequence specific primers can be used as RT primer. For nonamers and oligo $d(T)_{15}VN$ 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 a *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:

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

* Only if random nonamers or oligo $d(T)_{15}VN$ are used.

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

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 pipetting.

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 $MgCl_2$	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	12.75	-	-
Total Mix	**40 µl**		

* If using a qPCR Core kit
** If using a qPCR MasterMix

Add 40 µl of PCR reaction Mix to 10 µl of the 1rst strand reaction mix, or a dilution of it.

4.2- In case of a SYBR® green I assay

Component	Volume (µl)		Final concentration
Reaction buffer	5 *	25 **	1x
50 mM $MgCl_2$	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	16.25	5	-
Total Mix	**40 µl**		

* 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 of the 1rst strand reaction mix, or a dilution of it.

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

4.4- Add 10 µl or a dilution of the first strand reaction mix in your reaction vials / 96-well plate / 384-well plate. Add 5 µl of the template control plus 5 µl of water or buffer into your positive control vials/wells ; add 10 µl of water or buffer into your negative control vials/wells.

4.5- Add 40 µl of the PCR reaction mix to each vial/well. Close and mix gently on a stirrer or spin down. Ensure that no bubbles are present in the reaction vials/wells. Reaction set up can be performed 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, $MgCl_2$ 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.

- Troubleshooting Guide for qPCR and qRT-PCR (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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