EasyJOE Multiplex Master Mix

EasyJOE Multiplex Master Mix enables real-time amplification of single or multiple nucleic acid targets in easy to use One Step RT-qPCR Probe Master Mix format with the fast mode or standard cycling parameters of various thermocyclers.

EasyJOE Multiplex Master Mix contains both reverse transcriptase and DNA polymerase to amplify DNA and RNA targets. The control primers and probes are premixed to identify the RNA of internal control (Cat. No. E0833) as well as the DNA internal control (Cat. No. E0834). The RNA/DNA internal controls are available separately from EURx. The probe targeting the internal controls is labeled with JOE™ and detected in the JOE™/HEX™/VIC® channel.

EasyJOE Multiplex Master Mix contains ROX dye as a passive reference dye for use on the Applied Biosystems instruments, including the Applied Biosystems 7500, ViiA™ 7 and QuantStudio™Systems. EasyJOE Multiplex Master Mix is compatible with instrumentation that does not require ROX dye, including the Rotor-Gene® Q and Bio-Rad CFX96™.

	Cat. No. E0832-01 **100 reactions**	**Cat. No. E0832-02** **1 000 reactions**
EasyJOE Multiplex Master Mix	1.5 ml	10 x 1.5 ml

Storage and Handling

- Store at -20°C, secured from any sources of contaminating DNA or RNA.
- Avoid repeated thawing and freezing (>2), as this may reduce assay sensitivity. Freeze in aliquots if it is only to be used intermittently.
- Positive Control should be stored separately.

Internal Control

The Internal Control template, usable as extraction or amplification control, is available separately as IC-RNAJOE (Cat. No. E0833) and IC-DNAJOE (Cat. No. E0834). The internal control monitors the entire PCR workflow for the presence of inhibitors or other workflow issues, including extraction, reagent or instrument errors and failures.

- To use as an extraction control, add 5 µl of the IC-DNAJOE or IC-RNAJOE per sample to the lysis buffer before nucleic acid purification.
- To use as an amplification control, add 0.5 µl of the IC-DNAJOE or IC-RNAJOE per PCR reaction.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

The reference dye ROX

The passive ROX reference dye included in the **EasyJOE Multiplex Master Mix** enables fluorescence normalization in particular cyclers. The use of ROX dye is required for all Applied Biosystems real-time PCR cyclers and optional for Agilent cyclers. ROX compensates for differences in fluorescence signal between wells caused by small differences in reaction volume and fluorescence fluctuations. ROX is not involved in the PCR reaction and does not interfere with real-time PCR on any instrument.

Preparation of the sample

RNA from a swab, saliva, secretion fluids or environmental sample should be isolated using kits dedicated to viral RNA purification. Follow the instructions recommended by the kit manufacturer.

PRECAUTION:

Please protect EasyJOE Multiplex Master Mix and the prepared master mix from bright and direct light, as the probes are sensitive to light. Transparent vials and tubes are recommended to allow for visual inspection when ensuring proper mixing.

PROCEDURE:

Pipette reaction according to the table below. Keep all components on ice till placed in the thermocycler.

Component:	Amount:
EasyJOE Multiplex Master Mix	15 µl
Primers and probe(s) to target(s) of interest*	2 µl
Sample nucleic acid**	X µl
RNase-free Water	to 25 µl

*A primer concentration of 400 to 800 nM and a probe concentration of 200 nM per final reaction is recommended.

**For amplification control, add the appropriate IC-DNA/RNAJOE volume to the master mix and adjust volumes accordingly or exceed the reaction volume slightly.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

EasyJOE Multiplex Master Mix is designed to run on the fast mode. Run the thermal cycler program as indicated in table:

Step	Temperature	Time	Number of Cycles
Reverse Transcription*	50°C	10 min	1
Initial Denaturation	95°C	2 min	1
Denaturation Annealing/Extension/Data acquisition	95°C 60°C	5 s 30 s	40-45
Cooling	4°C	Indefinite	1

*Avoid this step if only DNA target(s) is used.

The denaturation time and the annealing/extension temperature and times can be changed per lab specific configuration. Carefully examine the amplification plot, adjusting the baseline and threshold values, where required. The target and/or IC should amplify within each PCR reaction. If both the target and IC fail to amplify in a reaction, repeat the reaction.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23