

# ExpressTaq™ One-Step RT-qPCR MasterMix Rev 03/21

(Catalog # M1512-100; 100 Rxns; Store at -20 °C)

## I. Introduction:

BioVision's ExpressTaq™ One-Step RT-qPCR MasterMix provides a convenient setup using the RT-qPCR Enzyme Mix and ExpressTaq™ 2X qPCR MasterMix for both highly sensitive and specific reverse transcription, and TaqMan probe-based real-time qPCR amplification in a single reaction tube. Our proprietary RT-qPCR Enzyme Mix contains stabilizers and enhancers that optimize the two reactions in a "single step". This offers the end user flexibility with RNA templates and primer selection, an efficient and easy-to-use set up, and a reliable alternative to conventional "two-step" sequential RT-qPCR. The MasterMix is suitable for SNP genotyping assays, gene expression analysis, microarray validation, and high throughput screening applications. ROX reference dye is provided separately, making it universally compatible with most qPCR instruments.

## II. Key Features:

- High Sensitivity
- Superior Performance
- Reverse transcription and qPCR in a single reaction tube
- TaqMan probe based qPCR amplification

## III. Applications:

- SNP genotyping assays, gene expression analysis, microarray validation, and high throughput screening applications

## IV. Contents:

Components	M1512-100 (100 Rxns)	Part Number
ExpressTaq™ qPCR MasterMix	1.25 ml	M1512-100-1
RT-qPCR Enzyme Mix	40 µl	M1512-100-2
ROX Reference Dye	15 µl	M1512-100-3
Nuclease-Free Water	1.0 ml	M1512-100-4

## V. User Supplied Reagents and Equipment:

- Pipettes, Pipette tips
- PCR tubes
- Nuclease free water
- Primers (forward and reverse)
- Total RNA or poly(A) + mRNA
- TaqMan probe
- QPCR instrument

## VI. Shipping and Storage Conditions:

The MasterMix is shipped in dry ice. All the components should be stored at -20 °C.

## VII. Protocol:

The Reactions should be assembled in an RNase-free environment. The use of "clean" pipettors designated for PCR and aerosol-resistant barrier tips are recommended.

The recommended amount of ROX Reference Dye to be added into the MasterMix may vary depending on the qPCR machine type. Please refer to the table in the selection guide for information on ROX and qPCR machine type.

- No ROX equipment: Not needed.
- Low ROX equipment: 1 µl/1.25 ml MasterMix.
- High ROX equipment: 11 µl/1.25 ml MasterMix.

1. Thoroughly thaw and mix the individual components before use and **assemble the reaction** on ice.

Component	Volume
2X ExpressTaq™ qPCR MasterMix	10 µl
RT-qPCR Enzyme Mix	0.4 µl
Forward Primer	Variable (100 - 500 nM)
Reverse Primer	Variable (100 - 500 nM)
TaqMan Probe	Variable (100 - 300 nM)
Total RNA or poly(A) + mRNA *	Variable (1 pg - 2 µg/rxn)
Nuclease-free water	up to 20 µl

\* Amplicon should be <150 bp in size.

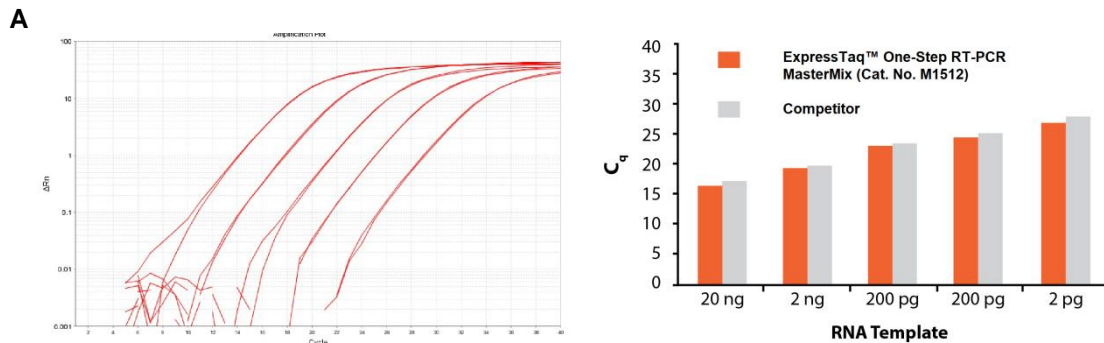
2. Gently mix the reaction components and briefly centrifuge.

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3. **Thermocycling conditions** for standard qPCR:

Step	Temperature	Duration		Cycles
		Standard	Fast	
cDNA Synthesis	50 °C	15 min	15 min	1
Enzyme Activation	95 °C	3 min	20 sec	1
Denaturation	95 °C	15 sec	1 sec	40
Annealing/Extension	60 °C	1 min	10 sec	

- Both poly(A) + mRNA and total RNA can be used for first-strand cDNA synthesis, but poly(A) + mRNA may give higher yields and improved purity of final products.
- For longer transcripts >9 kb, yields can be increased by incubating at 50 °C for 30-50 min.
- RNA samples must be free of genomic DNA contamination.
- To remove RNA complementary to the cDNA, add 1 µl of E. coli RNase H and incubate at 37 °C for 20 min.
- Use either the Standard or Fast qPCR program for your appropriate application.



**Fig. A. ExpressTaq™ One-Step RT-qPCR MasterMix** has excellent sensitivity and signal to noise ratio. 20 µl One-Step RT-qPCR reactions (20 ng, 2 ng, 200 pg, 2 pg of RNA per reaction) were carried out with BioVision’s ExpressTaq™ One-Step RT-qPCR MasterMix (Cat. No. M1512) for GAPDH amplification.

VIII. **Selection Guide For ROX and qPCR Machine Type:**

MACHINE TYPE	qPCR MACHINE
High ROX Machines	<ul style="list-style-type: none"> <li>ABI® 7000, 7300, 7700, 7900, 7900HT, StepOnePlus™, StepOne™, OpenArray, PRISM™ Sequencing Detection Series</li> <li>Biometra TOptical</li> <li>Fluidigm BioMark™</li> <li>Wafergene SmartChip System</li> <li>TianLong TL998 System</li> </ul>
Low ROX Machines	<ul style="list-style-type: none"> <li>ABI® 7500, 7500 Fast, Viia™ 7, QuantStudio, QuantStudio 3/5/6/7</li> <li>BioGene InSyte™</li> <li>Illumina Eco</li> <li>Analytikjena qTower Series</li> <li>Stratagene® Mx3000, Mx3005, Mx4000</li> </ul>
No ROX machines	<ul style="list-style-type: none"> <li>BioRad® CFX96, CFX384, Chromo4™, CFX Connect™, Opticon 2, MiniOpticon™</li> <li>Roche LightCycler® (480, 1536, Nano)</li> <li>MJ Research Opticon™, Opticon™ 2, Chromo® 4</li> <li>Eppendorf™ Realplex 4</li> <li>BioGene SynChron™</li> <li>Corbett Rotor-gene® (3000, 6200, 62H0, 6500, 65H0, 6600)</li> <li>Eppendorf Mastercycler® realplex (s, 4, 4s), Pro (S, 384), Nexus (gradient, eco, flat)</li> <li>Cepheid SmartCyler, GeneXpert</li> <li>Enigma™ ML</li> <li>Idaho LightScanner® (24, 32), RapidCycler®2, R.A.P.I.D (LT, LT Food), RAZOR EX, JBAIDS</li> <li>Qiagen Rotor-Gene™ (Q, 6000)</li> <li>Takara Dice™</li> <li>Thermo Scientific PikoReal</li> <li>DNA-Technology DT96, DTlite, DT-322</li> <li>Bioer LineGene (3310/3320, K FQD-48A, I, II, 9620, 9640, 9660, 9680)</li> <li>Bioneer Exicycler™</li> </ul>

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**IX. Related Products:**

<b>BioVision Product Name</b>	<b>Cat. No.</b>	<b>Sizes</b>
HiFidelity™ 2X PCR MasterMix	M1507	800 Rxns
ExpressTaq™ DNA Polymerase	M1504	400 Rxns
HiFidelity™ DNA Polymerase	M1505	400 Rxns
FireTaq™ DNA Polymerase	M1506	400 Rxns
ExpressTaq™ 2X PCR MasterMix	M1508	800 Rxns
HiFidelity™ One Step RT Kit	M1503	100 Rxns
ExpressTaq™ qPCR MasterMix	M1509	500 Rxns
Jade™ qPCR MasterMix	M1510	500 Rxns
Evo™ cDNA synthesis MasterMix	M1511	100 Rxns

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