Protocol for 1-step RT-qPCR

Using Lyo-ready Platinum II *Taq* Hot-Start DNA Polymerase and SuperScript Reverse Transcriptase

Invitrogen™ Lyo-ready Platinum™ II *Taq* Hot-Start DNA Polymerase (Cat. No. EP225SMP2)

Contents	Concentration	Quantity
Lyo-ready Platinum II *Taq* Hot- Start DNA Polymerase	20 U/µL	5,000 U
Lyo-ready Platinum II PCR Buffer	5X	6 x 10 mL
$MgCl_2$	50 mM	2 x 4 mL

Invitrogen™ Lyo-ready SuperScript™ Reverse Transcriptase, 1-Step RT-qPCR (Cat. No. EP215B2SMP1)

Contents	Concentration	Quantity
Lyo-ready SuperScript Reverse Transcriptase	200 U/µL	10,000 U

Storage conditions

- Store all contents at –20°C.
- Product is designed to withstand at least 10 freeze-thaw cycles.
- **Important:** 5X Lyo-ready Platinum II PCR Buffer may contain a precipitate. It should be warmed to room temperature and mixed thoroughly. Make sure the precipitate dissolves completely before use.

Required materials

- Template: viral RNA/DNA, human RNA/DNA, bacterial RNA/DNA
- Primers/probes
- dNTPs
- Reference dye (optional)
- Water, nuclease-free
- qPCR plastics

Product description

Lyo-ready Platinum II *Taq* Hot-Start DNA Polymerase

- Lyo-ready Platinum II *Taq* Hot-Start DNA Polymerase is an engineered *Taq* DNA polymerase that shows increased resistance to reaction inhibitors originating from sample materials or nucleic acid purification steps. The lyo-ready enzyme formulation offers the feasibility to lyophilize, while retaining all favorable properties of the standard enzyme preparation (with glycerol).
- The polymerase activity is blocked at ambient temperatures and restored after the initial denaturation step at 95°C. This automatic “hot start” provides increased sensitivity, specificity, and yield, while allowing reaction assembly at room temperature.

- Lyo-ready Platinum II *Taq* Hot-Start DNA Polymerase extends 1 kb in 15 seconds. The extension step can be prolonged without a negative effect on specificity.
- Like the standard *Taq* DNA polymerase, it has both 5′ to 3′ polymerase and 5′ to 3′ exonuclease activities but lacks 3′ to 5′ exonuclease activity.

Lyo-ready SuperScript Reverse Transcriptase, 1-Step RT-qPCR

- Lyo-ready SuperScript Reverse Transcriptase is an engineered version of Moloney murine leukemia virus (M-MuLV) reverse transcriptase with increased thermal stability and maintained RNase H activity.
- The enzyme can be used to synthesize first-strand cDNA at temperatures up to 55°C (optimally at 50°C), providing increased specificity and higher yields of cDNA.
- Lyo-ready Platinum II PCR Buffer is optimized to achieve the best performance for the reverse transcriptase and polymerase working as a pair in 1-step RT-qPCR. The buffer is also optimized for increased resistance to reaction inhibitors originating from sample material or nucleic acid purification steps.

Online resources

thermofisher.com/lyo-ready

For further information, contact
MDxenzymes@thermofisher.com

Protocol

Please follow the instructions below to prepare and run your 1-step RT-qPCR experiment.

Prepare 1-step RT-qPCR

The following example procedure shows the appropriate volumes for a single 20 µL 1-step RT-qPCR reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, then dispense the appropriate volumes into the qPCR plate before adding the template.

Steps

1. **Thaw reagents:** Thaw, mix, and briefly centrifuge each component before use.
2. **Prepare 1-step RT-qPCR master mix:** Combine the following components in a sterile, nuclease-free tube in the indicated order.
   Note: Consider the volumes of all components listed in steps 2 and 3 to determine the correct amount of water to reach your final reaction volume.

Component	Volume	Final concentration
Water, nuclease-free	to 20 µL	–
5X Lyo-ready Platinum II PCR Buffer	4 µL	1X
10 mM dNTP mix	1.2 µL	0.6 mM each (0.4–0.6 mM)
50 mM $MgCl_2$	3.2 µL	8 mM (6–8 mM)
10 µM forward primer	0.6 µL	0.3 µM (0.2–1 µM)
10 µM reverse primer	0.6 µL	0.3 µM (0.2–1 µM)
10 µM probe	0.4 µL	0.2 µM (0.15–0.5 µM*)
50 µM ROX reference dye (optional)	X µL	Varies (see table under “Optimization strategies”)
Lyo-ready Platinum II *Taq* Hot-Start DNA Polymerase, 20 U/µL	0.12 µL	0.12 U/µL (0.10–0.15 U/µL)
Lyo-ready SuperScript Reverse Transcriptase, 1-Step RT-qPCR, 200 U/µL	0.1 µL	1 U/µL (0.25–1.5 U/µL)**

* Follow general guidelines for primer design and primer concentration optimization for multiplex 1-step RT-qPCR.

** Concentrations of 0.25–0.5 U/µL are recommended for singleplex 1-step RT-qPCR. Higher concentrations of the enzyme are recommended for multiplex 1-step RT-qPCR.

Mix and then briefly centrifuge the master mix. Dispense the master mix into the qPCR plate.

3. **Add template DNA or RNA:** Add your template DNA or RNA to each qPCR plate well for a final reaction volume of 20 µL.

Component	Volume	Final concentration
Template RNA or DNA	X µL	Varies

Seal the plate with adhesive qPCR seals and centrifuge the plate.

4. **Run reactions in qPCR instrument:**

Step		Temperature	Recommended duration
Reverse transcription		50°C	5–15 min
RT reaction termination, initial denaturation, and polymerase activation		95°C	2 min
40 cycles	Denaturation	95°C	5 sec
	Annealing/ extension	60°C	15–30 sec*

* Keep in mind the specifications of the qPCR machine you use—15 sec might not be enough time for multiplex signal readouts for some machines.

Optimization strategies

Recommendations on ROX dye concentration for different qPCR instruments

Instrument	Final ROX dye concentration
Applied Biosystems™ 7300, 7900HT, StepOne™, StepOnePlus™, ABI PRISM™ 7000, and 7700 systems	300–500 nM
Applied Biosystems™ 7500, ViiA™ 7, and QuantStudio™ real-time PCR systems; Stratagene™ Mx3000P™, Mx3005P™, and Mx4000™ systems	30–50 nM
Bio-Rad™ iCycler iQ™, iQ™5, and MyiQ™, Opticon™, CFX96™, and CFX384™ systems; Roche™ LightCycler™ 480 and LightCycler™ 2.0 systems; Qiagen™ Corbett™ Rotor-Gene™ 3000 and 6000 systems; Eppendorf™ Mastercycler™ system; Cepheid™ SmartCycler™ system	Not required

Find out more at **thermofisher.com/lyo-ready**

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