

Titanium® One-Step RT-PCR Kit Protocol

PT3397-2

Table of Contents

I. Preparing an RT-PCR Master Mix.....	1
II. Setting Up the Reactions.....	1
III. Running the Reactions.....	2

NOTE: Please thoroughly read the User Manual (PT3397-1) before using this abbreviated protocol. This protocol is provided for your convenience, but is not intended for first-time users.

I. Preparing an RT-PCR Master Mix

Prepare a Master Mix as shown below. Prepare sufficient Master Mix for all of your reactions plus one additional reaction to ensure adequate volume.

- 5.0 µl 10X One-Step Buffer
- 1.0 µl 50X dNTP Mix
- 0.5 µl Recombinant RNase Inhibitor (40 units/µl)
- 25.0 µl Thermostabilizing Reagent
- 10.0 µl GC-Melt™
- 1.0 µl Oligo(dT) Primer
- 1.0 µl 50X Titanium *Taq* RT Enzyme Mix

43.5 µl Total Volume

II. Setting Up the Reactions

Set up reactions as shown below:

- 1–5.5 µl RNA sample (1 ng–1 µg)
- 1.0 µl PCR primer mix (45 µM each)
- 43.5 µl Master Mix
- x µl RNase-Free H₂O (add to 50 µl final volume)

50.0 µl Final Volume

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III. Running the Reactions

Commence thermal cycling using the following program. These parameters were optimized for amplifying the control 540-bp mouse β -actin fragment using a PE Biosystems DNA Thermal Cycler 480. This program can be used for hot-lid or non-hot-lid thermal cyclers.

- 50°C for 1 hr
- 94°C for 5 min
- 25–35 cycles^a:
 - 94°C 30 sec
 - 65°C 30 sec
 - 68°C 1 min^b
- 68°C for 2 min

^a Optimal number of cycles depends on transcript abundance and template complexity and must be determined empirically.

^b For experimental reactions, use 1–1.5 min of extension time per kb.

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