INSTRUCTION MANUAL

# One*Taq*® One-Step RT-PCR Kit

NEB #E5315S

30 reactions

Version 5.0_10/20

## Table of Contents



## Kit Components

*All kit components should be stored for one year at –20°C except where noted.*

| | |
|---|---|
| **One*Taq* One-Step Enzyme Mix**<br>ProtoScript® II Reverse Transcriptase, One*Taq* Hot Start DNA Polymerase, Murine RNase Inhibitor and stabilizers | 60 µl |
| **One*Taq* One-Step Reaction Mix**<br>Buffer containing 3.2 mM $MgCl_2$, 0.5 mM dNTPs each | 800 µl |
| **Quick-Load® One*Taq* One-Step Reaction Mix**<br>Colored buffer containing 3.2 mM $MgCl_2$, 0.5 mM dNTPs each, for direct gel loading | 800 µl |
| Nuclease-free $H_2O$ | 1.5 µl |

## Introduction

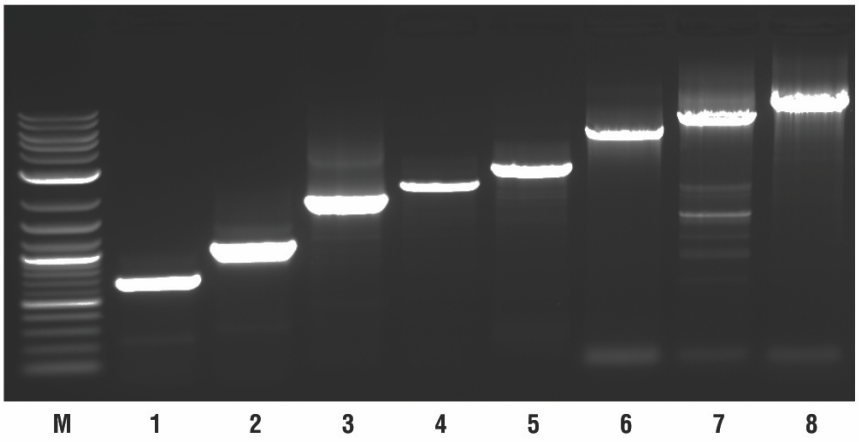
The One*Taq* One-Step RT-PCR Kit offers sensitive and robust end-point detection of RNA templates. cDNA synthesis and PCR amplification steps are performed in a single reaction using gene-specific primers, resulting in a streamlined RT-PCR protocol.

The kit combines three optimized mixes: One*Taq* One-Step Enzyme Mix, One*Taq* One-Step Reaction Mix and One*Taq* One-Step Quick-Load Reaction Mix. The Enzyme Mix combines ProtoScript II Reverse Transcriptase, Murine RNase Inhibitor and One*Taq* Hot Start DNA Polymerase. ProtoScript II Reverse Transcriptase is a mutant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. One*Taq* Hot Start DNA Polymerase is mixture of a Hot Start *Taq* DNA Polymerase combined with a proof-reading DNA polymerase, resulting in high-yield amplification with minimal optimization. The One*Taq* One-Step RT-PCR Kit is capable of amplifying long transcripts up to 9 kb in length.

Two optimized reaction mixes are included, One*Taq* One-Step Reaction Mix and Quick-Load One*Taq* One-Step Reaction Mix. The reaction mixes offer robust conditions for both cDNA synthesis and PCR amplification. The unique One*Taq* Quick-Load One-Step Reaction Mix contains additional dyes, offering color indication for reaction setup as well as direct gel loading.

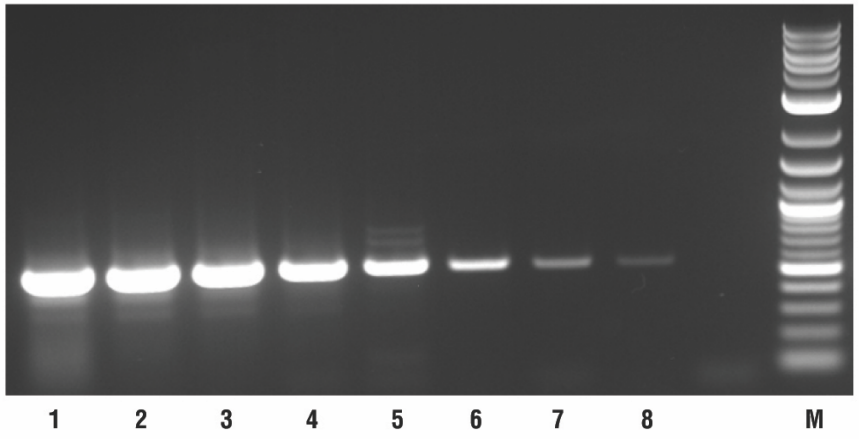
Both total RNA and mRNA can be used as template. The kit can detect a GAPDH target as low as 0.1 pg per reaction. It can routinely detect RNA targets up to 9 kb. The One*Taq* One-Step RT-PCR Kit is capable of multiplex detection of two or three targets.

**Figure 1: Detection of RNA templates of different length.**




*About 100 ng of Jurkat total RNA was used in 50 µl reactions following the standard protocol. The target sizes were Lane 1: 0.7 kb, Lane 2: 1.1 kb, Lane 3: 1.9 kb, Lane 4: 2.3 kb, Lane 5: 2.5 kb, Lane 6: 5.5 kb, Lane 7: 7.6 kb and Lane 8: 9.3 kb. The marker lane (M) contains Quick-Load 1 kb Plus DNA Ladder (NEB #N0469).*

**Figure 2: Sensitive detection of RNA down to 1 pg.**




*Serial dilution of HeLa total RNA (Lane 1: 1 µg, Lane 2: 100 ng, Lane 3: 10 ng, Lane 4: 1 ng, Lane 5: 100 pg, Lane 6: 10 pg, Lane 7: 1 pg, Lane 8: 0.1 pg) was used in 50 µl reactions following the Standard Protocol. The marker lane (M) contains Quick-Load 1 kb Plus DNA Ladder (NEB #N0469).*

## Quality Controls:

The performance of One*Taq* One-Step RT-PCR Master Mix is tested for sensitivity, multiplexing and product length. Using a GAPDH amplicon, the kit can detect 1 pg of Hela total RNA after 40 cycles. Multiplex detection of three targets is tested with amplicons of 0.5 kb, 0.6 kb and 0.7 kb. The length of cDNA achieved is verified by detection of a 9.3 kb amplicon of SMG1 phosphatidylinositol 3-kinase-related kinase gene after 40 cycles..

## Important Factors for Successful RT-PCR

### Template RNA

- High quality RNA is essential for sensitive target detection.
- For low abundance genes or long targets, more RNA template is required.
- RNA template should be free of any RNase contamination.
- Denaturation of RNA and primer at 65–70°C for 5 minutes may remove some secondary structures, thereby improving target detection.

## RNA-priming Choices

- We recommend using gene-specific primers for one-step RT-PCR. Design of gene-specific primers should follow best practices ("Guidelines for PCR Optimization with Thermophilic DNA Polymerases" at www.neb.com).
- For most reactions, a final primer concentration of 200–400 nM is optimal. For best results, we recommend titering primer concentrations in the range of 100–800 nM.
- If genomic DNA contamination is an issue, try to design primers located on different exons or at the exon-intron junction.

## Magnesium and dNTPs

- The magnesium concentration in the Reaction Mix is 3.2 mM (1.6 mM at final 1X concentration), which is optimal for most targets.
- The dNTP concentration in the reaction mix is 500 nM (250 nM at final 1X concentration), which is optimal for most targets.

## GC-rich Targets

- DMSO, up to 10%, can be included to improve the detection of GC-rich targets

## Reaction Conditions

- First strand cDNA synthesis can be carried out between 42°C to 55°C. As a starting point, we recommend incubation at 48°C for 15 minutes. Short targets can be completed with shorter incubation times. Longer incubations up to 1 hour may increase the cDNA yield.
- The pre-denature step at 94°C for 1 minute is sufficient to inactivate ProtoScript II Reverse Transcriptase.

| REACTION COMPONENTS | FINAL CONCENTRATION |
|---|---|
| Reaction Mix | 1X |
| Enzyme Mix | 1X |
| RNA Template | 1 µg-100 pg of total RNA<br>1 pg-100 ng of mRNA |
| Gene Specific Primers | 100-800 nM |

## Standard PCR Protocol

| CYCLE STEP | TEMP | TIME | CYCLES |
|---|---|---|---|
| Reverse Transcription | 48°C | 15-30 minutes | 1 |
| Initial Denaturation | 94°C | 1 minute | 1 |
| Denaturation | 94°C | 15 seconds | 40 |
| Annealing | 50-65°C | 30 seconds | |
| Extension | 68°C | 1 minute per kb | |
| Final Extension | 68°C | 5 minutes | 1 |
| Hold | 4°C | ∞ | 1 |

## One-Step RT-PCR Protocols

Quick-Load One*Taq* One-Step Reaction Mix can be used if direct gel loading is desired.

### Standard Protocol

Thaw system components and mix by inverting several times.

1. Mix the following components, except RNA, in sterile RNase-free microfuge tubes.

| REAGENT | VOLUME |
|---|---|
| One*Taq* One-Step Reaction Mix (2X) | 25 µl |
| One*Taq* One-Step Enzyme Mix (25X) | 2 µl |
| Gene-specific Forward Primer (10 µM) | 2 µl |
| Gene-specific Reverse Primer (10 µM) | 2 µl |
| Nuclease-free $H_2O$ | 19–x µl |
| Total RNA (up to 1 µg) | x µl |
| Total Volume | 50 µl |

2. Add RNA template last, and start reactions immediately, as follows:

| CYCLE STEP | TEMP | TIME | CYCLES |
|---|---|---|---|
| Reverse Transcription | 48°C | 15-30 minutes | 1 |
| Initial Denaturation | 94°C | 1 minute | 1 |
| Denaturation | 94°C | 15 seconds | 40 |
| Annealing | 50-65°C | 30 seconds | |
| Extension | 68°C | 1 minute per kb | |
| Final Extension | 68°C | 5 minutes | 1 |
| Hold | 4°C | ∞ | 1 |

### Alternative Protocol

If denaturation of template RNA is desired, use the following protocol:

1. Mix RNA sample and primers in sterile, RNase-free microfuge tubes.

| REAGENT | VOLUME |
|---|---|
| Total RNA (up to 1 µg) | x µl |
| Gene-specific reverse primer (10 µM) | 2 µl |
| One*Taq* One-Step Reaction Mix (2X) | 25 µl |
| Nuclease-free $H_2O$ | 19–x µl |
| Total Volume | 46 µl |

Denature for 5 minutes at 65°C. Put promptly on ice.

2. Add the following components to the tube:

| REAGENT | VOLUME |
|---|---|
| One*Taq* One-Step Enzyme Mix (25X) | 2 µl |
| Gene-specific Forward Primer (10 µM) | 2 µl |

3. Add tubes to the thermocycler, and run the following program:

| CYCLE STEP | TEMP | TIME | CYCLES |
|---|---|---|---|
| Reverse Transcription | 48°C | 15 minutes | 1 |
| Initial Denaturation | 94°C | 1 minute | 1 |
| Denaturation | 94°C | 15 seconds | 40 |
| Annealing | 50-65°C | 30 seconds | |
| Extension | 68°C | 1 minute per kb | |
| Final Extension | 68°C | 5 minutes | 1 |
| Hold | 4°C | ∞ | 1 |

## No-RT Negative Control Reaction

Mix the following components in a sterile RNase-free microfuge tube:

| REAGENT | VOLUME |
|---|---|
| One*Taq* One-Step Reaction Mix (2X) | 25 µl |
| Gene-specific Forward Primer (10 µM) | 2 µl |
| Gene-specific Reverse Primer (10 µM) | 2 µl |
| Hot Start One*Taq* DNA Polymerase (not provided) | 2 U |
| Nuclease-free $H_20$ | 19-x µl |
| Total RNA (up to 1 µg) | x µl |
| Total Volume | 50 µl |

Proceed with reaction conditions as follows:

| CYCLE STEP | TEMP | TIME | CYCLES |
|---|---|---|---|
| Reverse Transcription | 48°C | 15 minutes | 1 |
| Initial Denaturation | 94°C | 1 minute | 1 |
| Denaturation | 94°C | 15 seconds | 40 |
| Annealing | 50-65°C | 30 seconds | |
| Extension | 68°C | 1 minute per kb | |
| Final Extension | 68°C | 5 minutes | 1 |
| Hold | 4°C | ∞ | 1 |

## Troubleshooting

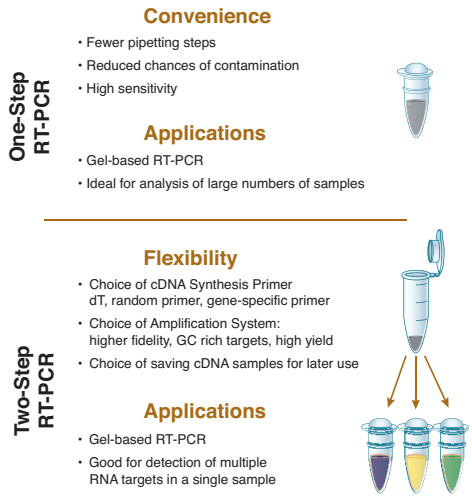
| OBSERVATION | POSSIBLE CAUSE(S) | SOLUTION(S) |
|---|---|---|
| Low yield of product | RNA is low quality | • Check the integrity of the RNA by denaturing agarose gel electrophoresis (2).<br>• RNA should have a minimum $A_{260}/A_{280}$ ratio of 1.7 or higher. Ethanol precipitation followed by a 70% ethanol wash can remove most contaminants such as EDTA and guanidinium. Precipitation with lithium chloride can remove polysaccharides (2).<br>• Phenol/chloroform extraction and ethanol extraction can remove contaminant proteins such as proteases (2). |
| | Not enough RNA | • RNA targets are present at low abundance; use 100 ng-1 µg total RNA or m RNA. |
| | Inefficient cDNA Synthesis | • Some target RNA may contain strong pauses for RT denature the RNA sample or redesign primers. |
| | PCR Condition is Not Optimal | • Optimize annealing temperature, increase cycle number, and/or design. |
| Low Specificity | Problematic Primers | • Re-design primers following guidelines of primer design. |
| | Amplification of larger genomic copy | • Design primers located on different exons or at the intron-exon junction. Pre-treat RNA samples with DNasel. |
| | PCR condition is not optimal | • Optimize annealing temperature, and/or increase cycle number |

## References


1. Liao, J. and Gong, Z. (1997) *Biotechniques*, 23, 368–370.
2. Sambrook, J. and Russel, D.W. (2001) Molecular Cloning: A Laboratory Manual (3rd Ed.) Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
3. Sellner, L N. and Turbett, G. R. (1998) *Biotechniques*, 25, 230–234.

## Frequently Asked Questions (FAQs)

**How do I choose between One-Step RT-PCR and Two-Step RT-PCR protocols?**
Both one-step and two-step RT-PCR protocols offer robust detection of RNA targets. Depending on your experimental design and goals, you may prefer one protocol over the other. Please refer to the following table:




**What's the difference between NEB #E6560 and NEB #E5315?**
Both kits use ProtoScript II Reverse Transcriptase for First Strand cDNA Synthesis. ProtoScript II Reverse Transcriptase is a mutant M-MuLV Reverse Transcriptase with reduced RNase H activity and increased thermostability. NEB #E6560 is a kit for first strand cDNA synthesis; its product can be used for downstream PCR or qPCR analysis. NEB #E5315 is a One-Step RT-PCR kit; its product is a specific dsDNA PCR product.

**How does One-Step RT-PCR kit protocol work for NEB #E5315?**
The One-Step RT-PCR reactions allow both cDNA synthesis and PCR amplification in the same reaction buffer. A thermocycler is programmed so that cDNA synthesis takes place first (48°C for 15 minutes), then reverse transcriptase is inactivated while Hot Start Taq Polymerase is activated (94°C for 1 minute), followed by 40 cycles of PCR amplification.

**Can I use a 25 µl reaction volume rather than a 50 µl reaction volume using NEB #E5315?**
Yes, you can use a 25 µl reaction by scaling down reagent volumes by half.

**What conditions should I use for cDNA synthesis?**
We recommend using a cDNA synthesis temperature in the range of 42°C to 55°C for 15–30 minutes. Routinely, we use 48°C for 15 minutes.

**Do I need to optimize the reaction components?**
The One*Taq* One-Step RT-PCR Kit standard reaction conditions (1.6 mM $MgCl_2$ and 250 µM dNTP at 1X) work well for most targets. You may be able to add more $MgCl_2$ or dNTPs to make your reactions work better, but in most cases re-designing your primers may be more effective.

**How much RNA template should I use?**
RNA template of high quality is critical for successful RT-PCR detection. Most targets can be routinely detected with inputs of 100 ng total RNA. We recommend using 10–100 ng of RNA per reaction, if possible. The more abundant your target RNA is, the less RNA template you all need to use.

**How should I design my primers?**
We recommend following general guidelines for PCR primer design: primers are generally 20–40 nucleotides long, with GC content of 40–60%, and primer pairs should not share complementary sequences at the 3′ end.

**What's the recommended primer concentration?**
For most targets, primers at 200 nM to 400 nM work well. You may want to titer the primer concentration to the range of 100 nM to 800 nM.

**How many PCR cycles should I do?**
We recommend 40 cycles for most targets. Some abundant RNA targets can be detected with as few as 25 cycles.

## Ordering Information

| NEB # | PRODUCT | SIZE |
|---|---|---|
| E5315S | One*Taq* One-Step RT-PCR Kit | 30 reactions |

COMPANION PRODUCTS

| NEB # | PRODUCT | SIZE |
|---|---|---|
| E6560S/L | ProtoScript II First Strand cDNA Synthesis Kit | 30/150 reactions |
| M0368S/L/X | ProtoScript II Reverse Transcriptase | 4,000/10,000/40,000 units |
| N0550S | Quick-Load Purple 1 kb Plus DNA Ladder | 250 gel lanes |
| M0314S/L | RNase Inhibitor, Murine | 3,000/15,000 units |
| M0481S/L/X | One*Taq* Hot Start DNA Polymerase | 200/1,000/5,000 units |
| N0447S/L | Deoxynucleotide (dNTP) Solution Mix | 40/80 µmol of each |

## Revision History

| REVISION # | DESCRIPTION | DATE |
|---|---|---|
| 1.0 | | 8/14 |
| 1.1 | | 11/15 |
| 2.0 | | 6/18 |
| 3.0 | Update to new manual format | 1/20 |
| 4.0 | Update legal text. | 3/20 |
| 5.0 | Update legal text | 10/20 |





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