Associated products

Product	Description	Pack Size	Cat No.
ISOLATE II RNA Mini Kit	Fast and efficient isolation of extremely pure total RNA from a variety of samples	10 Preps 50 Preps 250 Preps	BIO-52071 BIO-52072 BIO-52073
ISOLATE II Plant RNA Kit	Fast and efficient isolation of extremely pure total RNA from a variety of plant samples	10 Preps 50 Preps	BIO-52076 BIO-52077
TRIsure™	Quick isolation of high-quality RNA from a variety of sources for subsequent use in cDNA synthesis	100ml 200ml	BIO-38032 BIO-38033
Agarose	Molecular biology grade agarose	100g 500g	BIO-41026 BIO-41025

Technical support

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact Technical Support with details of reaction setup, cycling conditions and relevant data.

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Trademark and licensing information

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MyTaq™ One-Step RT-PCR Kit

Shipping: On dry/blue ice

Catalog numbers

Batch No.: See vial

Concentration: See vial

BIO-65048 25 Reactions

BIO-65049 100 Reactions

BIOLINE
A Meridian Life Science® Company

Store at –20 °C

Storage and stability:
MyTaq™ One-Step RT-PCR Kit is shipped on dry/blue ice. All kit components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended.

Expiry:
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Quality control:
MyTaq One-Step RT-PCR Kit and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Safety precautions:
Please refer to the material safety data sheet for further information.

Notes:
Research Use Only.

Description

MyTaq™ One-Step RT-PCR Kit has been formulated for highly reproducible first-strand cDNA synthesis and subsequent PCR in a single tube. A combination of the latest advances in buffer chemistry together with a reverse transcriptase and hot-start DNA polymerase system ensures that MyTaq One-Step RT-PCR Kit produces fast, highly-specific and ultra-sensitive one-step RT-PCR, from either total RNA or mRNA using gene-specific primers.

MyTaq One-Step Kit consists of a 2x MyTaq One-Step mix, as well as separate reverse transcriptase and RiboSafe RNase Inhibitor.

Kit components

Reagent	25 Reactions	100 Reactions
MyTaq One-Step mix (2x)	1 x 625 µL	2 x 1.25 mL
RiboSafe RNase Inhibitor (10u/µl)	1 x 25 µL	1 x 100 µL
Reverse transcriptase	1 x 12.5 µL	1 x 50 µL
DEPC-treated Water	1 x 1.8 mL	1 x 1.8 mL

General considerations

When handling RNA, it is important to use RNase-free plasticware and reagents. We also recommend performing RNA work in an RNase-free area. To help prevent any carry-over DNA contamination, we recommend that separate areas are maintained for reaction set-up, PCR amplification and any post-PCR gel analysis. It is essential that any tubes containing amplified DNA product are not opened in the reaction set-up area.

Primers: The sequence and concentration of the primers, as well as amplicon length, can be critical for specific amplification, yield and overall efficiency of any RT-PCR.

We strongly recommend taking the following points into consideration when designing and running your RT-PCR:

- The use of gene-specific primers is recommended for use with the MyTaq One-Step RT-PCR Kit. The use of oligo dT or random hexamers is not recommended with a One-Step RT-PCR set-up since this can result in the generation of non-specific products.
- use primer-design software, such as Primer3 (http://frodo.wi.mit.edu/primer3/) or visual OMP™ (http://dnasoftware.com/). Primers should have a melting temperature (Tm) of approximately 60°C
- final primer concentration of 400 nM is suitable for most RT-PCR, however, to determine the optimal concentration we recommend titrating in the range 0.1-1µM
- use an equimolar primer concentration

Template: It is important that the RNA template is intact and devoid of both DNA and contaminating inhibitors which can effect both reverse transcription and PCR. For high purity RNA, we recommend using the Bioline ISOLATE RNA Mini Kit (BIO-52043). RNA stocks and dilutions should be made in DEPC-treated Water (BIO-38030) to avoid any RNase-mediated degradation.

The recommended amount of template for one-step RT-PCR is dependent upon the type of RNA used. The following should be considered when using total RNA and isolated mRNA:

- **total RNA:** purified total RNA can be used in the range from 1pg to 1µg per 50µl reaction
- **mRNA:** purified mRNA can be used from 0.01pg per 50µl reaction

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3mM. In the majority of RT-PCR conditions, this is optimal for both the reverse transcriptase and the hot-start DNA polymerase. If necessary, we suggest titrating the $MgCl_2$ to a maximum of 5mM.

RT-PCR controls: It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no-RT control, by omitting the reverse transcriptase from the reaction.

Procedure

The following RT-PCR instructions are intended as a guideline for setting-up and running RT-PCR.

Reaction mix preparation: Before preparing an RT-PCR mastermix, ensure all reagents are mixed thoroughly. The volumes given below are based on a standard 50µl final reaction mix and can be scaled accordingly.

Kit Components:

Reagent	Volume	Final concentration
2x MyTaq One-Step Mix	25.0 µL	1x
Forward Primer (10µM)	2.0 µL	400 nM
Reverse Primer (10µM)	2.0 µL	400 nM
Reverse transcriptase	0.5 µL	-
RiboSafe RNase Inhibitor	1.0 µL	-
DEPC-H_2O	Up to 45 µL	-
Template	5µl	-
Final volume	**50 µL**	

Suggested RT-PCR conditions: The following RT-PCR conditions are suitable for the MyTaq One-Step RT-PCR Kit with the majority of amplicons.

Cycles	Temperature	Time	Notes
1	45 °C	20 min	Reverse transcription
1	95 °C	1 min	Polymerase activation
40	95 °C 60 °C 72 °C	10 s 10 s 30 s	Denaturation Annealing Extension

RT-PCR optimization: The following optimization may be necessary to improve the efficiency of some reactions:

- The reverse transcription reaction temperature can be increased from 45 °C to 50 °C for high GC-rich templates
- The reverse transcription time can be increased from 20 to 40 minutes for amplicons over 1 kb
- Increase the extension time of the PCR for target sequences longer than 1kb or if running multiplex RT-PCR

Troubleshooting guide

Problem	Possible Cause	Recommendation
No product on agarose gel	Activation time too short	Ensure MyTaq One-Step mix is activated for a minimum of 1min at 95 °C before cycling
	Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used
	Suboptimal primer design	Use primer design software or validated primers. Test primers on a control template
	Incorrect concentration of primers	Use primer concentrations between 100 nM and 1µM
	Template degraded	Re-isolate your template from the sample material or use freshly prepared template dilution. We recommend using the ISOLATE RNA kits for template preparation and DEPC-treated water for resuspension or dilution of the template Verify the integrity of RNA using agarose gel electrophoresis Ensure RNase inhibitor is added before addition of template
	Primers degraded	Do not use old diluted primer stock, use newly synthesized primers and store in TE (pH 8.5) rather than water. Avoid repeated freeze/thawing of stock solution
	Template contaminated with RT-PCR inhibitors	Further dilute template before PCR or purify template and resuspend it in DEPC treated water
	Template concentration too low	Increase concentration used
	Cycling conditions not optimal	Increase extension/annealing time, increase cycle number, reduce annealing temperature

Troubleshooting Guide (Continued)

Problem	Possible Cause	Recommendation
Non-specific amplification product AND / OR Primer-dimers	Non-specific priming	Use gene-specific primers rather than Oligo dT or random hexamers.
	Inefficient reverse transcription	Increase the reverse transcription temperature up to 48 °C
	Suboptimal primer design	Redesign primers using appropriate software, or use validated primers
	Primer concentration too high	Test dilution series of primer concentrations until primer-dimer/non-specific amplification products disappear
	Primer concentration too low	Use primer concentration between 100 nM and 1µM
	Primer annealing temperature too low	Increase PCR annealing temperature up to 65 °C or until primer-dimer/ non-specific amplification products disappear
	Template concentration too low	Increase template concentration
	Template concentration too high	Reduce template concentration until non-specific products disappear
	Extension time too long	Reduce extension time to determine whether non-specific products are reduced
Variability between replicates	Error in reaction set-up	Prepare large volume mastermix
	Air bubbles in reaction mix	Centrifuge reaction samples/plate prior to running RT-PCR
Low yield of PCR products	Inefficient reverse transcription	Increase the reverse transcription temperature up to 48 °C, and increase the RT incubation time
	Activation time too short	Ensure MyTaq One-Step mix is activated for a minimum of 1min at 95 °C before cycling
	Annealing temperature too high	Decrease annealing temperature in steps of 2 °C
	Extension time too short	Double extension time to determine whether the yield is affected
	Template concentration too low	Increase concentration if possible
	Template is degraded	Re-isolate template from sample material or use freshly prepared template dilution
	Suboptimal primer design	Redesign primers using appropriate software, or use validated primers
	Primer concentration too low	Increase concentration of primers in 100 nM increments