Thermophilic Reverse Transcriptase

Product Name and Catalog number

Thermophilic Reverse Transcriptase:
Taq-Standard-SYBR Green, Cat. # W140-TS
Taq-Fast-SYBR Green, Cat. # W140-TF
Taq-Probe-SYBR Green, Cat. # W140-TP
Size: 200U for 200 reactions (No ROX)

Intended Use

This reverse transcriptase or RNA-dependent DNA polymerase is used for real time-fluorescence RT-PCR with either TaqMan probe or SYBR Green dye. Research use only.

Please Choose a DNA polymerase and associated 5x RT-PCR Buffer before you start:

Depending on used DNA polymerases, the kit contains different buffers.

Table 1. Different DNA polymerases with related buffers

DNA polymerase	Fluorescence available	5x Buffer
Taq polymerase[a]	SYBR Green	**A**
Taq-Fast polymerase[b]	SYBR Green	**B**
Taq-Probe[c] polymerase	TaqMan Probe	**C**

Footnotes of Table 1

[a] *Taq* DNA polymerase is not included in the kit.

[b] *Taq*-Fast polymerase, Cat.# W148, can extend more than 300 bases with short PCR cycling program.

[c] *Taq*-Probe polymerase, Cat.# W145, a specially engineered enzyme for S-shaped curve, is not included.

Part I is for *Taq* DNA polymerase with Taq-Standard SYBR Green Dye, W140-TS, page 2-3.

Part II is for *Taq*-Fast polymerase with SYBR Green Dye, W140-TF, page 4-5.

Part III is for *Taq*-Probe polymerase with TaqMan probe, W140-TP, page 6-7.

Part I: Thermophilic Reverse Transcriptase

Thermophilic Reverse Transcriptase **for SYBR Green Dye together with *Taq* DNA polymerase**:

Cat.# **W140-TS**, 200U for 200 reactions x 20µl Taq-Standard-SYBR Green (No ROX)

Intended Use

This reverse transcriptase or RNA-dependent DNA polymerase is used for real-time-fluorescent RT-PCR with SYBR Green dye.

Features

- The reverse transcriptase is a thermophilic type A polymerase (US patent pending) with optimal temperatures at 60-62°C (Tables 1-2).
- The RTase is easily heat-inactivated at ≥90°C for 1min.
- The enzyme efficiently synthesizes a complementary DNA strand on RNA template from a gene-specific primer, *≤1 unit per 20µL of* singleplex *reaction.*
- The enzyme reversely-transcribes single digit copies of target RNA molecules consistently.
- The concentrations of the primers are variable depending on assay designs and thermocycling protocols (Table 4).
- The preferable PCR product size is ≤150bp.
- It is particularly suitable for one-step real-time qualitative and quantitative RT-PCR.

Table 1. Enzyme properties

Reverse transcriptase activity	Yes
5'-3' DNA polymerase activity	Yes
5'-3' exonuclease activity	Yes
3'-5' exonuclease activity	No
Incorporation of modified nucleotides	Yes, *e.g.*, dUTP, fluorescence dye-labeled dNTPs

Table 2. Optimal parameters

Optimal RTase amount	0.5-1U/20µl reaction
Optimal temperature	60-62°C
Optimal incubation time	5-10min
Heat-Inactivated	≥90°C for 2min
Mg_2 concentration	1.5mM
dNTP concentration	Each 200uM
Primer concentration	Each ≥0.15µM, depending on primer design and thermocycling
Product size	Preferred ≤150bp

Unit Definition

One unit of the enzyme catalyzes incorporation of 1nmol of deoxyribonucleotides into polynucleotide in 10min.

Production Source

E. coli strain

Table 3. Kit Contents

Component	Amount
RTase, 10U/µl	200U/500U/ 2500U
5x Buffer **A**	1mL/2x1mL/10mL
User manual	1

Ship and Store

The kit can be transported below 4°C for up to 3 days. It can be stored at -20°C for 24 months.

Setup Reaction and Thermocycling

1. Thaw 5x Buffer and other reaction components at room temperature, mix each component, centrifuge, and then place on ice.

2. Determine the total volume for the number of reactions, add 5-10% extra volume, and prepare assay mix of all components except RNA template. Mix the assay mix, centrifuge, and then place on ice.

3. Aliquot the assay mix into PCR tubes or plates.

4. Add RNA template to PCR tubes or plate.

5. Seal tubes with flat, optically transparent caps or seal plates with optically transparent film.

6. Mix and then briefly centrifuge the tubes or plate.

7. Program PCR instrument with indicated thermo-cycling protocol.

8. Load PCR tubes or plates and start to run.

9. Perform data analysis according to the PCR instrument instructions.

Table 4. Set up a 20µl of RT-PCR reaction.

Component	Amount or final concentration
5x Buffer **A**	4ul
dNTPs	Each 200uM
Target specific primers[a]	Each 0.15-0.2uM

RTase, 10U/μl	0.5-1U
***Taq* polymerase**[c]	1U
RNA template[d]	As low as single digit copies of target RNA
Nuclease-free H_2O	To a total volume of 20μl

Footnotes of Table 4

[a] The primer T_m should be designed ≥60°C, preferably between 62°C to 65°C, using primer3 software for high efficiency and specificity.

[d] RNA templates should be extracted by a qualified silica-based kit and eluted with low EDTA TE buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0-8.3).

Table 5. Compatible instruments

RT-PCR Instrument	ROX required by instrument	Passive dye setup
Bio-Rad® iQ™5, CFX96, CFX384, Opticon Roche Lightcycler® Qiagen Rotor-Gene™ Eppendorf Mastercycler® Cepheid® SmartCycler®	Not recommended	Not necessary
Applied Biosystems® 7500, 7500 Fast, QuantStudio™, ViiA7™, Agilent Mx™	Low ROX (50nM final concentration)	Turn on ROX passive reference dye button
Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™	High ROX (500nM final concentration)	Turn on ROX passive reference dye button

Table 6. Standard thermocycling protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	2min	1
III	95°C	10sec	35-40
	60°C, signal acquisition	60sec	

Table 7. Three-Step Thermocycling Protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	2min	1
III	95°C	10sec	35-40
	60°C	30sec	
	68-72°C, signal acquisition	30sec	

Footnotes of Tables 6 and 7

The three-step thermocycling protocol in Table 7 increases overall DNA polymerase activity by 50%, a more effective protocol than Table 6.

The primer concentration used in Tables 6 and 7 is typically 0.15-0.2uM.

Part II: Thermophilic Reverse Transcriptase

Product Name and Catalog Number

Thermophilic Reverse Transcriptase **for SYBR Green Dye together with *Taq*-Fast polymerase**

Cat.#: **W140-TF**, 200U for 200 reactions x 20µL (No ROX)

Intended Use

This reverse transcriptase or RNA-dependent DNA polymerase is used for real time-fluorescence RT-PCR with SYBR Green dye.

Features

- The reverse transcriptase is a thermophilic type A polymerase (US patent pending) with optimal temperatures at 60-62°C (Tables 1-2).
- The RTase is easily heat-inactivated at ≥90°C for 1min.
- The enzyme efficiently synthesizes a complementary DNA strand on RNA template from a gene-specific primer, *≤1 unit per 20µL of singleplex reaction.*
- The enzyme reversely-transcribes single digit copies of target RNA molecules consistently.
- The concentrations of the primers are variable depending on assay designs and thermocycling protocols (Table 4).
- The preferred PCR product size is ≤150bp.
- It is particularly suitable for one-step real-time qualitative and quantitative RT-PCR.

Table 1. Enzyme properties

Reverse transcriptase activity	Yes
5'-3' DNA polymerase activity	Yes
5'-3' exonuclease activity	Yes
3'-5' exonuclease activity	No
Incorporation of modified nucleotides	Yes, *e.g.*, dUTP, fluorescence dye-labeled dNTPs

Table 2. Optimal parameters

Optimal RTase amount	≤1U/20µl reaction
Optimal temperature	60-62°C
Optimal incubation time	5-10min
Heat-Inactivated	≥90°C for 1min
Mg_2 concentration	1.5mM
dNTP concentration	Each 200uM
Primer concentration	Each ≥0.15µM, depending on primer design and thermocycling
Product size	Preferred ≤150bp

Unit Definition

One unit of the enzyme catalyzes incorporation of 1nmol of deoxyribonucleotides into polynucleotide in 10min.

Production Source

E. coli strain

Table 3. Kit Contents

Component	Amount
RTase, 10U/µl	200U/500U, 2500U
5x Buffer **B** (SYBR Green)	1mL/2x1mL/ 10mL
User manual	1

Notes: *Taq*–Fast polymerase, Cat.# W148, which can extend more than 300 bases with short cycling program, and it is not included in the kit.

Transportation and Storage

The kit can be transported below 4°C for up to 3 days. It can be stored at -20°C for 24 months.

Setup Reaction and Thermocycling

1. Thaw 5x Buffer and other reaction components at room temperature, mix each component, centrifuge, and then place on ice.
2. Determine the total volume for the number of reactions, add 5-10% extra volume, and prepare assay mix of all components except RNA template. Mix the assay mix, centrifuge and then place on ice.
3. Aliquot the assay mix into PCR tubes or plates.
4. Add RNA template to PCR tubes or plate.
5. Seal tubes with flat, optically transparent caps or seal plates with optically transparent film.
6. Mix and then briefly centrifuge the tubes or plate.
7. Program PCR instrument with indicated thermo-cycling protocol.
8. Load PCR tubes or plates and start to run.
9. Perform data analysis according to the PCR instrument instructions.

Table 4. Set up a 20µl of RT-PCR reaction.

Component	Amount or final concentration
5x Buffer **B**	4ul
dNTPs	Each 200uM
Target specific primers[a]	Each ≥0.15uM
RTase, 10U/µl	≤1U
***Taq*-Fast polymerase[b]**	1U
RNA template[c]	As low as single digit copies of target RNA
Nuclease-free H_2O	To a total volume of 20µl

Footnotes of Table 4

[a] The primer T_m should be designed ≥60˚C, preferably between 62˚C to 65˚C, using primer3 software for high efficiency and specificity.

[b] *Taq*-Fast polymerase, which can extend more than 300 bases with a short cycling program, is not included in the kit.

[c] RNA templates should be extracted by a qualified silica-based kit and eluted with low EDTA TE buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0-8.3).

Table 5. Compatible instruments

RT-PCR Instrument	ROX required by instrument	Passive dye setup
Bio-Rad® iQ™5, CFX96, CFX384, Opticon Roche Lightcycler® Qiagen Rotor-Gene™ Eppendorf Mastercycler® Cepheid® SmartCycler®	Not recommended	Not necessary
Applied Biosystems® 7500, 7500 Fast, QuantStudio™, ViiA7™, Agilent Mx™	Low ROX (50nM final concentration)	Turn on ROX passive reference dye button
Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™	High ROX (500nM final concentration)	Turn on ROX passive reference dye button

Table 6. Standard thermocycling protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	2min	1
III	95°C	10sec	35-40
	60°C, signal acquisition	60sec	
IV	60°C to 95°C	Various	1

Table 7. Three-Step Thermocycling Protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	2min	1
III	95°C	10sec	35-40
	60°C	30sec	
	68-72°C, signal acquisition	30sec	
IV	68-72°C to 95°C	Various	1

Footnotes of Tables 6 and 7

The three-step thermocycling protocol in Table 7 increases overall DNA polymerase activity by 50%, a more effective protocol than Table 6.

The primer concentration used in Tables 6 and 7 is typically 0.15-0.2uM.

Table 8. Fast thermocycling protocol

Stage	Temperature	Period	Number of cycles
I	60°C	5-10min	1
II	95°C	1min	1
III	95°C	5sec	35-40
	60°C, signal acquisition	30sec	
IV	60°C to 95°C	Various	1

Footnotes of Table 8

The product size for the fast two-step thermocycling protocol is preferred to be less than 90bp.

The primer concentration used is typically between 0.4uM and 0.9uM.

Thermophilic Reverse Transcriptase

Product Name and Catalog number

Thermophilic Reverse Transcriptase for **TaqMan probe with *Taq*-Probe polymerase**

Cat. #: **W140-TP**, 200U for 200 reactions x 20µL (No ROX)

Intended Use

This reverse transcriptase or RNA-dependent DNA polymerase is used for real-time-fluorescent RT-PCR with TaqMan probe.

Features

- The reverse transcriptase is a thermophilic type A polymerase (US patent pending) with optimal temperatures at 60-62°C (Tables 1-2).
- The RTase is easily heat-inactivated at ≥90°C for 1min.
- The enzyme efficiently synthesizes a complementary DNA strand on RNA template from a gene-specific primer, *≤1 unit per 20µL of* singleplex *reaction.*
- The enzyme reversely-transcribes single digit copies of target RNA molecules consistently.
- The concentrations of the primers are variable depending on assay designs and thermocycling protocols (Table 4).
- The preferred PCR product size is ≤150bp.
- It is particularly suitable for one-step real-time qualitative and quantitative RT-PCR.

Table 1. Enzyme properties

Reverse transcriptase activity	Yes
5'-3' DNA polymerase activity	Yes
5'-3' exonuclease activity	Yes
3'-5' exonuclease activity	No
Incorporation of modified nucleotides	Yes, *e.g.*, dUTP, fluorescence dye-labeled dNTPs

Table 2. Optimal parameters

Optimal RTase amount	Singleplex: 1 pair of primers: 0.5-1U/20µl reaction
	Multiplex: 2-4 pairs of primers: 1-2U/20µl reaction
Optimal temperature	60-62°C
Optimal incubation time	5-10min
Heat-Inactivated	≥90°C for 2min
Mg_2 concentration	1.5-3mM
dNTP concentration	Each 200uM
Primer concentration	Each ≥0.15µM, depending on primer design and thermocycling
Probe concentration	Each 0.15-0.25uM
Product size	Preferred ≤150bp

Unit Definition

One unit of the enzyme catalyzes incorporation of 1nmol of deoxyribonucleotides into polynucleotide in 10min.

Production Source

E. coli strain

Table 3. Kit Contents

Component	Amount
RTase, 10U/µl	200U/500U/ 2500U
5x Buffer **C**	1mL/2x1mL/10mL
User manual	1

Notes: *Taq*-Probe polymerase, Cat. # W145, a specially engineered enzyme for S-shaped curve, is not included.

Transportation and Storage

The kit can be transported below 4°C for up to 3 days. It can be stored at -20°C for 24 months.

Setup Reaction and Thermocycling

1. Thaw 5x Buffer and other reaction components at room temperature, mix each component, centrifuge and then place on ice.

2. Determine the total volume for the number of reactions, add 5-10% extra volume, and prepare assay mix of all components except RNA template. Mix the assay mix, centrifuge and then place on ice.

3. Aliquot the assay mix into PCR tubes or plate.

4. Add RNA template to PCR tubes or plate.

5. Seal tubes with flat, optically transparent caps or seal plates with optically transparent film.

6. Mix and then briefly centrifuge the tubes or plate.

7. Program PCR instrument with indicated thermo-cycling protocol.

8. Load PCR tubes or plates and start to run.

9. Perform data analysis according to the PCR instrument instructions.

Table 4. Set up a 20µl of RT-PCR reaction

Component	Amount or final concentration
5x Buffer **C**	4ul
dNTPs	Each 200uM
Target specific primers[a]	Each 0.15-0.2uM
Probes[b]	Each 0.15-0.25uM
RTase, 10U/µl	Singleplex: 0.5-1U
	Multiplex: 1-2U
***Taq*-Probe polymerase[c]**	Singleplex: 2U
	Multiplex up to four templates: 4U
RNA template[d]	As low as single digit copies of target RNA
Nuclease-free H_2O	To a total volume of 20µl

Footnotes of Table 4

[a] The primer T_m should be designed ≥60°C, preferably between 62°C to 65°C, using primer3 software for high efficiency and specificity.

[b] The probe's T_m should be designed between 70-75°C.

[c] *Taq*-Probe polymerase, a specially engineered enzyme for S-shaped curve, is not included (*Taq*-Probe Polymerase, Cat W145-TP).

[d] RNA templates should be extracted by a qualified silica-based kit and eluted with low EDTA TE buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0-8.3).

Table 5. Compatible instruments

RT-PCR Instrument	ROX required by instrument	Passive dye setup
Bio-Rad® iQ™5, CFX96, CFX384, Opticon Roche Lightcycler® Qiagen Rotor-Gene™ Eppendorf Mastercycler® Cepheid® SmartCycler®	Not recommended	Not necessary
Applied Biosystems® 7500, 7500 Fast, QuantStudio™, ViiA7™, Agilent Mx™	Low ROX (50nM final concentration)	Turn on ROX passive reference dye button
Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™	High ROX (500nM final concentration)	Turn on ROX passive reference dye button

Table 6. Standard thermocycling protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	2min	1
III	95°C	10sec	35-40
	60°C, signal acquisition	60sec	

Table 7. Three-Step Thermocycling Protocol

Stage	Temperature	Period	Number of cycles
I	60°C	10min	1
II	95°C	2min	1
III	95°C	10sec	35-40
	60°C	30sec	
	68-72°C, signal acquisition	30sec	

Footnotes of Tables 6 and 7

The three-step thermocycling protocol in Table 7 increases overall DNA polymerase activity by 50%, a more effective protocol than Table 6.

The primer concentration used in Tables 6 and 7 is typically 0.15-0.2uM.

Related Products

- Thermophilic Reverse Transcriptase, Cat. # W140
- *Taq*-Probe Polymerase, Cat.# W145
- *Taq*-Fast Polymerase, Cat.# W148
- 1-Step 2X Fast RT-PCR Master Mix-SYBR Green, Cat.# W147
- 1-Step 2X RT-PCR Master Mix-TaqMan Probe, Cat.# W143
- 1-Step 2X Multiplex RT-PCR Master Mix-TaqMan Probe, Cat. # W146
- 1-Step 2X Super Multiplex RT-PCR Master Mix-TaqMan Probe, Cat. # W149