YUANZAN LIFE SCIENCES

ENGLISH PRODUCT MANUALS

Ten translated technical product documents

01	YZ-204002	DNA Ladder 2000
02	YZ-301002	2x Taq Plus Master Mix
03	YZ-301008	2x Fast Pfu Master Mix (Quick Load)
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05	YZ-301012	Taq-Plus PCR Master Mix (2x)
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10	YZ-302016-2	First-Strand cDNA Synthesis SuperMix for qPCR

Source: User-supplied Yuanzan Chinese-language technical manuals.

Translation only. Product development / QA approval required before official release.

November 4, 2026

DNA Ladder 2000

Catalog No.: YZ-204002
Product Information / Instructions for Use — English Translation

1. Product Description

DNA Ladder 2000 consists of **six linear double-stranded DNA fragments** and is designed for estimating DNA fragment sizes during **agarose gel electrophoresis**. The 750 bp band is provided as a bright reference band at approximately **30 ng/μL**; the other bands are approximately **10 ng/μL** each, helping users identify bands during gel analysis.

The product already contains **1× DNA Loading Buffer** and can be loaded directly onto an agarose gel. It is **not recommended for polyacrylamide gel electrophoresis**.

2. DNA Fragment Composition

100 bp, 250 bp, 500 bp, 750 bp (bright band), 1,000 bp, and 2,000 bp.

Electrophoresis Figure (From the Source)

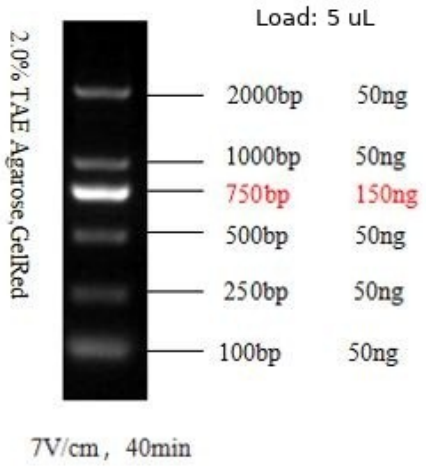
The original document contains an agarose-gel illustration with a **5 μL** loading volume, indicating a **150 ng** bright 750 bp band and approximately **50 ng** for each other band at that volume. The photographed bands are labeled 2,000, 1,000, 750, 500, 250, and 100 bp. Experimental notation: **2.0% TAE agarose gel, 7 V/cm, 40 min**.

3. Recommendations for Use

1. Load **3–5 μL** per gel well, or approximately **1 μL per millimeter of well width**.
2. Use **1–2% agarose**, an electric field strength of **4–10 V/cm**, and **1× TAE** or **0.5× TBE** electrophoresis buffer.
3. DNA bands can be visualized using ethidium bromide (EB) or other nucleic acid dyes. Follow the instructions for the chosen dye. If band smearing occurs with a precast gel containing a dye, reducing the dye concentration may help. Bands can be viewed under UV illumination using the relevant laboratory safeguards.
4. When using the separately supplied **5× DNA Loading Buffer**, dilute it to **1×**; the source specifies a ratio of **DNA sample : 5× DNA Loading Buffer = 4 : 1**. Mix and load directly.

Important: DNA Ladder 2000 itself already includes **1× Loading Buffer**. No additional loading buffer is needed for the ladder.

4. Electrophoresis Figure



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The original PDF shows a representative gel with a bright 750 bp band. See the original source image when comparing migration behavior and intensity. Visual results depend on gel concentration, dye and operating conditions.

5. Storage

Store at **−20°C ± 5°C**. After thawing, hold at **2–8°C**. Avoid repeated freeze–thaw cycles.

6. Product Contents

Component	YZ-204002A	YZ-204002B
DNA Ladder 2000	500 µL	5 × 500 µL
5× DNA Loading Buffer	500 µL	5 × 500 µL

Prepare the 5× buffer at 1× when used separately. The DNA Ladder 2000 already contains 1× Loading Buffer.

7. Precautions

1. Ensure reagents are completely thawed and mixed before use.
2. Use high-quality, freshly prepared agarose gels and replace electrophoresis buffer as needed.
3. For gels stained with ethidium bromide, the source recommends stopping electrophoresis when the bromophenol blue indicator has traveled roughly two-thirds of the gel length to avoid weakening the smaller bands.
4. When using a newer nucleic acid stain such as GelRed, the source recommends post-electrophoresis staining.
5. Agarose percentage affects DNA fragment separation. Higher percentage gels favor shorter fragments; lower percentage gels favor larger fragments. The source gives these approximate guidance ranges:

Agarose Concentration	DNA Fragment Range
0.5%	1,000–30,000 bp
0.7%	800–12,000 bp
1.0%	500–10,000 bp
1.2%	400–7,000 bp
1.5%	200–3,000 bp
2.0%	50–2,000 bp

1. TBE generally provides better separation of short fragments, whereas TAE may be useful for larger fragments and certain DNA conformations, as discussed in the source. Choose buffer according to the experiment.
2. The product includes **xylene cyanol** and **bromophenol blue** tracking dyes. The source gives approximate migration positions of **2,000 bp** and **300–400 bp**, respectively.

8. Troubleshooting

Q1. Why are DNA ladder bands missing or too faint?

Gel preparation: An unsuitable concentration, poor agarose quality, or improper storage may affect results. The source recommends 1–2% agarose, dissolving agarose fully, and using fresh gels. For precast dye approaches, allow the molten gel to cool briefly before dye addition and to set for 30–60 minutes; do not rely on overnight-stored gels when fresh gel is available.

Staining approach: Excessively short or long post-staining can lead to poor staining; the source recommends about **30 minutes** of soaking, adjusted to the dye instructions. With highly sensitive dyes such as GelRed, reducing the amount of DNA ladder or using post-staining may improve results.

Electrophoresis: Use **4–10 V/cm**, 1× TAE or 0.5× TBE; excessive voltage may overheat buffer and distort bands.

Q2. Why are bands curved, wavy, or dumbbell-shaped?

- Use fresh gels, or follow the source storage approach in electrophoresis buffer if immediate use is not possible.
- The source notes that dye type and premixing may change migration; with GelRed, post-staining may help, or reduce ladder loading volume.
- Confirm electrophoresis voltage is stable and within the recommended **4–10 V/cm** range.

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2× Taq Plus Master Mix

Catalog No.: YZ-301002
Product Information / Instructions for Use — English Translation

1. Product Description

2× Taq Plus Master Mix is a ready-to-use premix containing Taq Plus DNA polymerase, dNTPs, $MgCl_2$, and reaction buffer at **2× concentration**. The formulation is optimized for long-fragment DNA PCR amplification. According to the manufacturer, the mix offers increased amplification length, favorable fidelity, faster amplification, and efficient reaction performance, and is suitable for **1–20 kb** fragments.

For PCR setup, add template and primers and dilute the mix to **1×**. The product simplifies handling and helps reduce contamination opportunities. The resulting PCR products have a **3′ A overhang** and can be cloned into T vectors.

2. Product Features (Manufacturer Statements)

1. **High efficiency:** Amplification of up to **20 kb** using lambda DNA template, as demonstrated in the original manual.
2. **Sensitivity:** Claimed higher sensitivity than conventional Taq polymerase; see the original example figure.
3. **Stability:** The source states amplification performance remains unaffected after dozens of freeze–thaw cycles, **30 days at 4°C**, or **one week at room temperature** under the reported conditions.
4. **Convenience:** Required PCR reagents are provided together, allowing reaction setup within minutes.

Translation qualification: These are claims as printed in the supplied product instructions and are not independently validated in this translation.

3. Intended Applications

1. General applications that use a 2× Taq Master Mix.
2. PCR amplification requiring higher sensitivity.
3. Long-fragment PCR amplification (up to **20 kb**, as described).

4. Quality Control

The manufacturer reports testing for absence of residual exogenous nuclease activity and *E. coli* DNA by qPCR. It also reports successful amplification of a single-copy gene from human genomic DNA.

5. Storage

Store at **−20°C**.

6. Product Contents

Component	YZ-301002A	YZ-301002B
2× Taq Plus Master Mix	5 × 1 mL	(5 × 1 mL) × 5

Source printing note: The first catalog number is printed as “Z-301002A” in one source table; it is normalized here to the product-series prefix **YZ-** for the translation filename and heading. Confirm the catalog identifier before external publication.

7. Recommendations

PCR products generated with this mix have a **3′ A overhang**, permitting insertion into T vectors through the appropriate cloning workflow.

8. Precautions

1. Thaw completely before use to prevent uneven ion concentrations.
2. Choose the PCR cycle count for the experiment. Too few cycles may result in insufficient product; too many may increase mutations and nonspecific amplification.
3. Select annealing temperature according to primer Tm. Temperatures that are too low may produce nonspecific bands; temperatures that are too high may prevent the target from amplifying.

9. Directions for Use

1. Typical 50 µL Reaction

Component	Amount
2× Taq Plus Master Mix	25 µL
Forward primer	0.2–1.0 µM final
Reverse primer	0.2–1.0 µM final
Template (plasmid)	1–50 ng
OR Template (genomic DNA)	10 ng–1 µg
ddH_2O	To 50 µL

Final Mg^{2+} concentration stated in the source: 2 mM.

2. Typical PCR Cycling Program — Fragments Under 3 kb

Step	Temperature	Duration	Cycles
Initial denaturation	94°C	90 s	1
Denaturation	94°C	20 s	30
Annealing	50–60°C	20 s	30
Extension	72°C	60 s/kb	30
Final extension	72°C	5 min	1
Hold	4°C	Hold	1

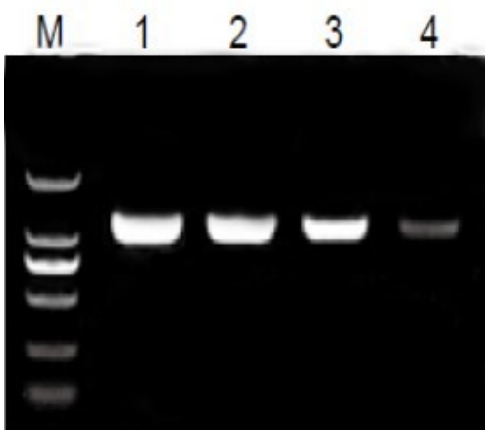
3. Typical PCR Cycling Program — Fragments 3 kb or Longer

The source recommends **primers of at least 30 bp** for this program.

Step	Temperature	Duration	Cycles
Initial denaturation	94°C	5 min	1
Denaturation	94°C	5 s	30
Annealing/extension	68°C	60 s/kb	30
Final extension	72°C	5 min	1
Hold	4°C	Hold	1

10. Application Example

In a 50 µL reaction, the manufacturer illustrates amplification of a **1.1 kb** fragment from mouse genomic DNA using **100 ng, 10 ng, 1 ng, and 0.1 ng** template inputs.



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Gel lanes: M = DNA Ladder 2000; Lane 1 = 100 ng; Lane 2 = 10 ng; Lane 3 = 1 ng; Lane 4 = 0.1 ng. The original gel photograph is included in the source PDF.

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2× Fast Pfu Master Mix (Quick Load)

Catalog No.: YZ-301008
Product Information / Instructions for Use — English Translation

1. Product Description

2× Fast Pfu Master Mix (Quick Load) contains Fast Pfu DNA polymerase, dNTPs, $MgCl_2$, reaction buffer, optimization agents, and stabilizers, premixed at a **2× concentration**. The product is optimized for cloning-related DNA amplification and is described as a high-fidelity reagent with an error rate of **1.3×10^{-6}** (manufacturer claim). To set up a PCR reaction, add template and primers and dilute to 1×.

Compared with conventional Pfu polymerase, the manufacturer describes faster amplification, higher sensitivity, and stronger inhibitor tolerance, with applications including **1–20 kb** fragments. Loading dye is already included, so amplified products can be loaded directly for gel electrophoresis after cycling.

2. Intended Applications

PCR applications requiring greater fidelity or longer amplification length, including:

- Cloning PCR
- DNA fragment assembly
- Introduction of mutations
- Whole-gene synthesis

3. Product Features (Manufacturer Statements)

1. **High fidelity:** The source claims fidelity equivalent to Pfu polymerase and approximately **50-fold higher than conventional Taq**.
2. **Sensitivity:** Amplification of a target fragment from **0.05 ng** human genomic DNA template, as stated in the manual.
3. **Speed:** Fast Pfu is described as several times faster than standard Pfu, extending over 1 kb at **72°C for 30 seconds** under the tested conditions; see example gel in the original PDF.
4. **Convenience:** Required PCR components are premixed, simplifying setup.

Claim note: The figures and comparisons above are translated manufacturer claims; the PDF does not provide the detailed independent method needed to verify all of them.

4. Recommendations

Fast Pfu PCR products are **blunt-ended** and do **not** contain a 3′ A overhang.

Except when using the **NovoRec® PCR one-step directional cloning kit (Cat. No. NR005)** cited in the source, the manual describes these cloning options:

1. Phosphorylate primer 5′ ends before PCR or phosphorylate the PCR product, then ligate into a blunt-ended vector.
2. Add a 3′ A overhang to the PCR product before ligation into a T vector.
3. Because the 3′→5′ proofreading exonuclease activity may partially degrade primers from their 3′ ends, use primers of approximately **20–30 nucleotides** and prepare reactions on ice to reduce this issue.

5. Quality Control

The manufacturer states that no residual exogenous nuclease activity or *E. coli* DNA was detected by the stated test method and that a single-copy gene could be amplified from human genomic DNA.

6. Storage

Store at **−20°C**.

7. Product Contents

Component	YZ-301008A	YZ-301008B
2× Fast Pfu Master Mix (Quick Load)	1 mL	5 × 1 mL

8. Directions for Use

1. Standard 50 µL Reaction

Component	Amount
2× Fast Pfu Master Mix (Quick Load)	25 µL
Forward primer	0.2–1.0 µM final
Reverse primer	0.2–1.0 µM final
Plasmid template	1–50 ng
OR Genomic DNA template	10 ng–1 µg
ddH_2O	To 50 µL

Final Mg^{2+} concentration stated in the source: 2 mM.

2. Recommended Cycling Program — Fragments Under 3 kb

Step	Temperature	Duration	Cycles
Initial denaturation	94°C	90 s	1
Denaturation	94°C	20 s	30
Annealing	50–60°C	20 s	30
Extension	72°C	30 s/kb	30
Final extension	72°C	5 min	1
Hold	4°C	Hold	1

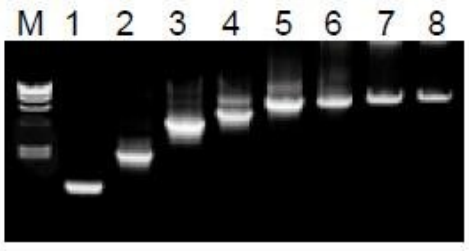
3. Recommended Cycling Program — Fragments 3 kb or Longer

The source recommends primer lengths of **at least 30 bp**.

Step	Temperature	Duration	Cycles
Initial denaturation	94°C	5 min	1
Denaturation	94°C	5 s	30
Annealing/extension	68°C	30 s/kb	30
Final extension	72°C	5 min	1
Hold	4°C	Hold	1

9. Application Example

The manual shows amplification of **1–20 kb** fragments using lambda DNA as the template in a 50 µL reaction.



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Gel lanes: M = lambda/HindIII DNA marker; Lane 1 = 1 kb; Lane 2 = 2 kb; Lane 3 = 4 kb; Lane 4 = 6 kb; Lane 5 = 8 kb; Lane 6 = 10 kb; Lane 7 = 12 kb; Lane 8 = 20 kb. See the gel photograph in the source PDF.

10. Precautions

1. Thaw completely before use to prevent uneven ionic concentration.
2. For high-GC templates, the source recommends raising denaturation temperature to **98°C**.
3. For high-GC templates, the source recommends using **PCR Universal Enhancer I (Cat. No. E055)** or adding **5–8% final DMSO**.

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2× Taq-AS PCR Mix (+Dye)

Catalog No.: YZ-301010
Product Information / Instructions for Use — English Translation

Storage and Transport

Store at **−20°C**.

Product Contents

Component	Pack Size
2× Taq-AS PCR Mix (+Dye)	5 × 1 mL

Product Description

2× Taq-AS PCR Mix (+Dye) is a premixed PCR reagent designed for convenient reaction setup. Add template and primers, then ddH_2O to obtain the required final **1**× concentration. The ready-to-use formulation reduces handling steps and contamination opportunities. PCR products contain a **3′ A overhang** and can be used for T/A cloning after purification.

The mix contains a selected **Taq-AS (Advanced Strong) DNA Polymerase** variant. The source describes efficient amplification of DNA fragments **up to 5 kb**, favorable tolerance to inhibitors, and amplification approximately **three times faster than conventional Taq DNA polymerase**, helping reduce extension time.

Unlike fast Taq polymerases based on fusion-protein designs, the source describes Taq-AS as more similar in use to wild-type Taq, with fewer problems such as diffuse or trailing bands or changes in product size.

The PCR mix contains two dyes, enabling PCR products to be loaded directly onto an electrophoresis gel without additional loading buffer. Two tracking bands, **purple and yellow**, appear during electrophoresis. The source states the dyes do not affect PCR efficiency, but recommends purifying PCR products before absorbance- or fluorescence-based optical analyses.

Quality Control

Endonuclease Activity Test

A 50 µL reaction containing **25 µL** 2× Taq-AS PCR Mix (+Dye) and **200 ng** supercoiled plasmid DNA was incubated at **37°C for 4 hours**. Agarose gel electrophoresis showed that less than **10%** of the plasmid DNA converted to nicked or linear forms.

Nonspecific Nuclease Activity Test

A 50 µL reaction containing **25 µL** of 2× Taq-AS PCR Mix (+Dye) and **15 ng** double-stranded DNA fragment was incubated at **37°C for 16 hours**. Agarose gel electrophoresis showed no change in the double-stranded DNA substrate.

Directions for Use

1. Standard PCR Reaction Setup (Prepare on Ice)

Component	Volume	Final Concentration
2× Taq-AS PCR Mix (+Dye)	25 µL	1×
Forward primer (10 µM)	1–2 µL	0.2–0.4 µM
Reverse primer (10 µM)	1–2 µL	0.2–0.4 µM

Template DNA	x µL	—
ddH_2O	To 50 µL	—

Notes:

1. Thaw fully to avoid uneven ion concentrations.
2. Recommended final primer concentration: **0.2–0.4 µM**; adjust within **0.1–1 µM** where needed.
3. For a 50 µL reaction, the source recommends **10–400 ng** for genomic DNA, or **10 pg–20 ng** for plasmid or viral DNA. Optimal input varies by template.

2. Three-Step PCR Cycling Program

Step	Temperature	Duration	Cycles
Initial denaturation	94°C	3–5 min	1
Denaturation	94°C	30 s	30–35
Annealing	55–65°C	30 s	30–35
Extension	72°C	20–40 s/kb	30–35
Final extension	72°C	5 min	1

3. Two-Step PCR Cycling Program (Targets ≥3 kb)

Step	Temperature	Duration	Cycles
Initial denaturation	94°C	3–5 min	1
Denaturation	94°C	30 s	30–35
Annealing/extension	68°C	30 s/kb	30–35
Final extension	72°C	5 min	1

For colony PCR, the source recommends **an initial denaturation time of at least 5 minutes** to help disrupt cells.

Troubleshooting

Problem	Possible Cause	Suggested Solution
No bands for either positive control or test sample	Unsuitable PCR reaction conditions	Optimize PCR conditions using gradient PCR.
No bands for either positive control or test sample	Reagent inactivity due to improper storage	Store at −20°C and avoid repeated freeze–thaw cycles. For frequent use, short-term storage at 4°C is described.
No bands for either positive control or test sample	Primer design problem	Redesign and check primers.
Positive control has target band; sample has no or weak band	Reagents have lost activity	Use fresh reagents.
Same	Excessive tissue lysate input	Increase reaction volume or decrease lysate input.
Same	Degraded DNA due to improper or prolonged storage of the lysate	Hold lysate at 4°C for 2–3 days at most as described; use freshly prepared lysate when possible.
Same	Unsuitable template volume	Optimize template input within 1–10% of total reaction volume.
Same	Insufficient cycling	Increase cycles to approximately 35–40; complex templates may require 5–10 more cycles than purified DNA.
Nonspecific amplification	Annealing temperature too low or cycle count/primer/template concentration too high	Raise annealing temperature or reduce cycling/primer/template input.
Nonspecific amplification	Primer mismatch	Redesign PCR primers.
Nonspecific amplification	Reaction assembly at high temperature or extended delay before amplification	Assemble the reaction at low temperature and start PCR promptly.

Negative control produces target band	Contaminated tools or reagents	Use the contamination-prevention measures described in the source; prevent target-sequence carryover and splashing.
Negative control produces target band	Cross-contamination between specimens	Use one sampling instrument for each sample or follow the source cleaning procedure, including repeated rinsing with **2% sodium hypochlorite solution** and wiping off residual liquid. Apply current validated laboratory safety procedures.

Manufacturer: Shanghai Yuanzan Life Science Co., Ltd.
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Taq-Plus PCR Master Mix (2×)

Catalog No.: YZ-301012
Product Information / Instructions for Use — English Translation

Storage and Transport

Store at **−20°C**.

Product Contents

Component	Pack Size
Taq-Plus PCR Master Mix (2×)	5 × 1 mL

Product Description

Taq-Plus PCR Master Mix (2×) is a ready-to-use PCR premix formulated with Taq DNA polymerase. It contains reaction buffer, dNTPs, and other components required for PCR. The premixed format simplifies and speeds up reaction setup and helps reduce contamination during PCR handling. To prepare a PCR reaction, add the template and primers to the appropriate volume of Taq-Plus PCR Master Mix (2×), then add ddH_2O to obtain a final 1× master mix concentration.

According to the manufacturer's instructions, the mix can amplify DNA fragments up to **5 kb** and offers favorable amplification specificity and template compatibility. PCR products contain a 3′ A overhang and may be used for T/A cloning after purification.

Quality Control

Endonuclease Activity Test

A 50 µL reaction containing 25 µL Taq-Plus PCR Master Mix and 200 ng supercoiled plasmid DNA was incubated at 37°C for 4 hours. Agarose gel electrophoresis showed that less than 10% of the plasmid DNA was converted into nicked or linear forms.

Nonspecific Nuclease Activity Test

A 50 µL reaction containing 25 µL Taq-Plus PCR Master Mix and 15 ng double-stranded DNA fragment was incubated at 37°C for 16 hours. Agarose gel electrophoresis showed no change in the double-stranded DNA substrate.

Directions for Use

1. Standard PCR Reaction Setup

Reagent	Volume	Final Concentration
Taq-Plus PCR Master Mix (2×)	25 µL	1×
Forward primer (10 µM)	1–2 µL	0.2–0.4 µM
Reverse primer (10 µM)	1–2 µL	0.2–0.4 µM
Template DNA	x µL	—
ddH_2O	To 50 µL	—

Notes:

1. A final primer concentration of 0.2–0.4 µM is recommended. If performance is unsatisfactory, optimize within 0.1–1 µM.

2. The optimal template amount depends on template type. For a 50 µL reaction, the recommended range is generally **10–400 ng** for genomic DNA and **10 pg–20 ng** for plasmid or viral DNA.

2. Standard PCR Cycling Program

Step	Temperature	Duration	Cycles
Initial denaturation	94°C	3–5 min	1
Denaturation	94°C	30 s	30–35
Annealing	55–65°C	30 s	30–35
Extension	72°C	30–60 s/kb	30–35
Final extension	72°C	5 min	1

Notes:

1. These initial denaturation conditions are suitable for most amplification reactions. For complex templates, such as microbial suspensions or colonies (especially yeast), the initial denaturation time may be increased to 10 minutes.
2. For target fragments no longer than 2 kb, an extension rate of 30 s/kb is recommended. For targets longer than 2 kb, use 60 s/kb.
3. When using a yeast-cell suspension as the PCR template, a target length of no more than 2.5 kb is recommended. For larger targets, pretreat yeast cells to disrupt the cell wall.

Troubleshooting

Problem	Possible Cause	Suggested Solution
No band for either the positive control or test sample	Unsuitable PCR mixture or conditions	Optimize conditions using gradient PCR.
No band for either the positive control or test sample	Reagent inactivity caused by improper storage	Store at −20°C; avoid repeated freeze–thaw cycles. For frequent use, short-term storage at 4°C is possible.
No band for either the positive control or test sample	Primer design problem	Redesign and evaluate primers.
No band for the test sample, or a weak band, while the positive control shows the target band	Loss of reagent activity after improper or prolonged storage	Use fresh reagent.
Same	Excessive amount of tissue lysate	Increase the reaction volume or reduce the lysate volume.
Same	Degraded genomic DNA due to improper or prolonged lysate storage	Lysate may be held at 4°C for 2–3 days; use freshly prepared lysate whenever possible.
Same	Unsuitable template input	Optimize template volume within 1–10% of the reaction volume.
Same	Insufficient PCR cycles	Increase to approximately 35–40 cycles; complex templates may require 5–10 more cycles than purified DNA templates.
Nonspecific amplification	Annealing temperature too low or cycle count, primer concentration, or template concentration too high	Increase annealing temperature; reduce cycle count, primer concentration, or template concentration.
Nonspecific amplification	Primer mismatch	Redesign PCR primers.
Nonspecific amplification	Reaction assembled at excessively high temperature or left standing too long before cycling	Assemble on a cool surface and begin cycling promptly.
Target band in negative control	Contaminated tools or reagents	Follow the source manual's contamination-control procedures and prevent splashing or carryover of target material.

Target band in negative control	Cross-contamination between specimens	Use a separate sampling tool for each specimen, or follow the source procedure for dipping the sampler tip into **2% sodium hypochlorite solution**, rinsing repeatedly, and wiping off residual liquid. Apply current validated laboratory safety procedures.

Translation note: This document reproduces the source instructions. For contamination-control procedures, apply the laboratory's current validated SOP rather than treating a general troubleshooting statement as universal practice.

Manufacturer: Shanghai Yuanzan Life Science Co., Ltd.
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Taq Visual SYBR qPCR Premix (Universal)

Catalog No.: YZ-301013
Product Information / Instructions for Use — English Translation

Storage and Transport

For long-term storage, keep at **−20°C**, protected from light. After thawing, the product can remain stable at **4°C for one month** when protected from light. Avoid repeated freeze–thaw cycles whenever possible.

Product Contents

Component	Pack Size (M)
Taq Visual SYBR qPCR Premix (Universal)	4 × 1.25 mL
10× Dilution Buffer	1 mL

Product Description

Taq Visual SYBR qPCR Premix (Universal) is a **2× premix for SYBR Green I dye-based real-time quantitative PCR**. It contains all the qPCR components except primers and DNA template, simplifying setup and reducing handling time and contamination risk. The core enzyme is antibody-mediated hot-start Taq DNA polymerase, used with an optimized buffer system and PCR-enhancing factors. The manufacturer describes high specificity, amplification efficiency, suppression of nonspecific amplification, and reliable quantification over a broad template concentration range.

The product contains a universal reference dye intended to provide compatibility with many qPCR instruments without additional reference dye adjustment; instrument-specific compatibility should be confirmed.

The premix includes **blue dye**, and the 10× Dilution Buffer contains **yellow dye**. When template prepared using the Dilution Buffer is added to the premix, the color changes **from blue to green**, enabling visual tracking of template addition.

Directions for Use

1. Template Dilution for Visual Pipetting Tracking

If pipetting tracking is desired, use the Dilution Buffer to prepare the template. Otherwise, the Dilution Buffer may be omitted.

Initial DNA Template Form	Use of 10× Dilution Buffer	Final Dilution Buffer Concentration in Template
Solid	Dilute the 10× buffer to 1× using ddH_2O and dissolve the DNA in the resulting 1× buffer	1×
Solution	If necessary, dilute the template with ddH_2O; add 1 µL of 10× Dilution Buffer to each 9 µL of template	1×

Caution: Incorrect use of Dilution Buffer may affect qPCR results.

2. Recommended qPCR Reaction Setup

Reagent	Volume	Final Concentration
Taq Visual SYBR qPCR Premix	10 µL	1×
Forward primer (10 µM)	0.4 µL	0.2 µM
Reverse primer (10 µM)	0.4 µL	0.2 µM

DNA template	x µL	10–200 ng per 20 µL reaction (as specified)
Nuclease-free water	To 20 µL	—

Notes:

1. The recommended final primer concentration is 0.2 µM, adjustable within 0.1–1 µM.
2. When using Dilution Buffer for pipetting tracking, limit template volume to **2–5 µL per 20 µL reaction**. Below 2 µL, color development may be weak; above 5 µL, Dilution Buffer components may interfere with qPCR.
3. For undiluted cDNA, whether or not it contains 1× Dilution Buffer, the template volume must not exceed **one-tenth of the reaction volume** (2 µL in a 20 µL reaction).
4. Optimal template input depends on sample type and target copy number; dilution series may be used as needed.

3. qPCR Cycling Conditions (Adjust for Instrument)

Two-step program

Step	Temperature	Time	Cycles
Initial denaturation	95°C	30 s	1
Denaturation	95°C	10 s	40
Annealing/extension	60°C	30 s	40
Melting curve	Instrument default acquisition program	—	1

Three-step program

Step	Temperature	Time	Cycles
Initial denaturation	95°C	30 s	1
Denaturation	95°C	10 s	40
Annealing	55–65°C	10 s	40
Extension	72°C	30 s	40
Melting curve	Instrument default acquisition program	—	1

Choose annealing and extension temperatures according to primer Tm. For amplicons of 200 bp or less, the annealing/extension step may be shortened to 15 seconds; account for the qPCR instrument's minimum fluorescence acquisition time.

4. Assay Optimization

Primer concentration: Within a 0.1–1.0 µM final concentration range, lower concentrations may improve specificity while potentially reducing amplification efficiency.

Cycling program: To improve specificity, consider a two-step program or higher annealing temperature. To improve amplification efficiency, consider a three-step program or longer extension time.

5. Primer Design Guidelines

1. Target amplicon length: **80–200 bp**.
2. Primer length: **18–25 bases**.
3. Forward/reverse primer Tm difference: no more than 1°C; target Tm **58–62°C**.
4. GC content: **40–60%**.
5. Distribute A/G/C/T bases evenly; avoid continuous T/C or A/G stretches, particularly at 3′ ends. The terminal 3′ base is preferably G or C.
6. Avoid intraprimer and forward/reverse primer complementarity.
7. Check primer specificity using **NCBI BLAST**.

Troubleshooting

Symptom	Possible Cause	Suggested Action
Amplification curve is not smooth	Weak fluorescence signal after software correction	Check whether the dye in the mix has degraded; use appropriate fluorescence-compatible qPCR consumables.
Amplification curve breaks or decreases	High template concentration; baseline end point exceeds Ct	Reduce the baseline end point to approximately Ct − 4, then reanalyze.
Sudden signal drop in individual wells	Bubbles in the reaction tube	Ensure the mix is completely dissolved; avoid vortexing; tap gently and briefly centrifuge to remove bubbles. The original manual also suggests extending initial denaturation to 10 minutes.
No amplification curve	Too few cycles	Set approximately 40 cycles; more cycles can increase background signals.
No amplification curve	Fluorescence acquisition step omitted or wrongly configured	In a two-step program, collect during annealing/extension; in a three-step program, collect during extension at 72°C, as specified.
No amplification curve	Degraded primers	Check primer integrity by PAGE if necessary.
No amplification curve	Low template concentration	Reduce the dilution factor or evaluate a higher-concentration template.
No amplification curve	Degraded template	Prepare fresh template and repeat.
Late Ct value	Low amplification efficiency	Increase primer concentration, consider three-step cycling, or redesign primers.
Late Ct value	Low or degraded template	Reduce dilution or prepare a new template.
Late Ct value	Amplicon too long	Use an 80–200 bp target amplicon.
Late Ct value	PCR inhibitors introduced with the template	Increase template dilution or prepare a purer template.
Signal in no-template control	Contaminated reaction components	Replace water, primers, tips, PCR tubes, or mix as needed; prepare reactions in a clean workstation.
Signal in no-template control	Primer dimers or other nonspecific amplification	Evaluate melting curves; the source notes that late signals after cycle 35 may occur, and recommends optimizing primer design/concentration or cycling conditions.
Multiple melting peaks	Poor primer specificity or excessive primer concentration	Redesign primers or lower concentration.
Multiple melting peaks	Genomic DNA contamination in cDNA	Treat extracted RNA with DNase (e.g., dsDNase), or use intron-spanning primers.
Poor replicate consistency	Pipetting error	Use accurate pipettes and suitable tips; increase template addition volume where appropriate; increase qPCR reaction volume.
Poor replicate consistency	Low template concentration	Reduce template dilution.
Poor replicate consistency	Temperature differences across instrument wells	Calibrate the qPCR instrument periodically.

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NovoScript® miRNA First-Strand cDNA Synthesis and SYBR qPCR Kit

Catalog No.: YZ-302002
Product Information / Instructions for Use — English Translation

1. Product Description

This kit contains the reagents required for miRNA testing and is suitable for samples containing miRNA, including miRNA preparations, total RNA, and small RNAs. First-strand cDNA is synthesized through poly(A) tailing and reverse transcription using NovoScript® miRNA RT Enzyme Mix and 2× miRNA RT Reaction Mix. Subsequent miRNA qPCR is performed with 2× miRNA SYBR qPCR SuperMix.

Poly(A) tailing and reverse transcription are combined within the first-strand cDNA synthesis workflow. NovoScript® miRNA RT Enzyme Mix contains poly(A) polymerase and reverse transcriptase. The 2× miRNA RT Reaction Mix contains the components needed for tailing and reverse transcription; add the miRNA-containing template and water to perform the reaction. This simplifies handling and reduces contamination opportunities.

The 2× miRNA SYBR qPCR SuperMix uses SYBR Green I dye-based fluorescence detection and contains hot-start DNA polymerase, an optimized reaction buffer, dNTPs, SYBR Green I, and other reagents. The kit includes **ROX Reference Dye I and II** for use according to the qPCR instrument and **Universal miRNA primer R**, so only the relevant miRNA-specific forward primer needs to be designed when this universal reverse primer is used.

2. Product Features

1. Reagents for miRNA detection are supplied in one kit; reverse transcription and qPCR are carried out as separate optimized reactions.
2. Poly(A) tailing and reverse transcription are integrated into the cDNA synthesis step, simplifying workflow.
3. The universal poly(A)-tailing reverse transcription approach allows different miRNAs to be investigated from the same RNA template, helping conserve sample.
4. Premixed cDNA synthesis and qPCR reagents reduce reagent preparation steps and handling time.
5. The source specifies a total RNA template input range of **10 pg–5 μg**.

3. Storage

Store at **−20°C**.

4. Kit Contents

Component	YZ-302002A (20 reactions)	YZ-302002B (40 reactions)
NovoScript® miRNA RT Enzyme Mix	20 μL	40 μL
2× miRNA RT Reaction Mix	200 μL	400 μL
Universal miRNA primer R (10 μM)	50 μL	100 μL
2× miRNA SYBR qPCR SuperMix	1 mL	2 × 1 mL
ROX Reference Dye I (ROX I)	50 μL	100 μL
ROX Reference Dye II (ROX II)	50 μL	100 μL
RNase-free water	1 mL	2 × 1 mL

Choose whether ROX is required, and which reference dye to use, according to the real-time PCR instrument.

5. Precautions

1. Gently invert all reagents to mix before use; minimize foam and briefly centrifuge.
2. Prevent RNase contamination throughout the procedure.

3. Avoid repeated freeze–thaw cycles.
4. Prepare reactions and add components on ice.
5. Use high-quality RNA template to ensure effective reverse transcription.

ROX Reference Dye Selection (Instrument Examples Listed in the Source)

ROX I: ABI 5700, 7000, 7300, 7700, 7900/7900 HT/7900 HT Fast, StepOne/StepOnePlus, and other instruments.

ROX II: ABI 7500/7500 Fast, ViiA 7, Q5/Q6, QuantStudio 6/7 Flex; Stratagene MX4000/MX3500P/MX3000P; and other instruments.

No ROX required (listed examples): Bio-Rad CFX96, CFX384, iCycler iQ/iQ5, My iQ5, MiniOpticon, Opticon, Chromo4; Eppendorf Mastercycler realplex; Cepheid SmartCycler; Illumina Eco qPCR; Roche LightCycler 480; Thermo PikoReal; Qiagen/Corbett Rotor-Gene models.

These lists reflect the source manual and should be checked against the current instrument instructions.

6. Procedure

A. First-Strand cDNA Synthesis

Thaw reagents, mix, briefly centrifuge, and place on ice. Prepare the reaction in the order shown.

Reagent	Volume / Amount
Total RNA or miRNA template	Total RNA: 10 pg–5 μg; or miRNA template
2× miRNA RT Reaction Mix	10 μL
NovoScript® miRNA RT Enzyme Mix	1 μL
RNase-free water	To 20 μL

Mix gently and briefly centrifuge.

Step	Temperature	Duration
Poly(A) tailing and cDNA synthesis	39°C	60 min
Reaction termination	85°C	5 min

Notes:

1. Recommended total RNA input is **0.01–2 μg**; up to **5 μg** may be used depending on miRNA abundance.
2. For high-GC or structured RNA, the source recommends combining template with RNase-free water, incubating at **65°C for 5 min**, cooling on ice, then adding other reagents.
3. The reverse transcription product may be used directly for qPCR or stored at **−20°C** if not used immediately.
4. If reverse transcription uses a high template concentration, the source recommends diluting cDNA **1- to 50-fold** for subsequent qPCR to achieve suitable Ct values.

B. qPCR Amplification of First-Strand cDNA

The kit contains **Universal miRNA primer R (10 μM)**. You may design the target-specific forward primer for use with this reverse primer or design target-specific forward and reverse primers following the guidance below.

Standard 20 μL qPCR reaction

Reagent	Volume
2× miRNA SYBR qPCR SuperMix	10 μL
Forward primer (10 μM)	0.4 μL
Reverse primer (10 μM)	0.4 μL
cDNA template	x μL
ROX (if required by instrument)	0.4 μL
RNase-free H_2O	To 20 μL

Two-step cycling

Step	Temperature	Duration / Cycles
Initial denaturation	95°C	1 min
Denaturation	95°C	5 s × 40–45 cycles
Annealing/extension	60°C	30 s × 40–45 cycles
Melting curve	According to instrument	—

Three-step cycling

Step	Temperature	Duration / Cycles
Initial denaturation	95°C	1 min
Denaturation	95°C	5 s × 40–45 cycles
Annealing	60°C	20 s × 40–45 cycles
Extension	72°C	30 s × 40–45 cycles
Melting curve	According to instrument	—

Notes: The Universal miRNA primer R has a stated Tm of **63°C**; aim for a target-specific forward primer Tm of **60–65°C**. Primer specificity may be checked using miRBase and a sequence search tool.

7. miRNA-Specific Primer Design Guidelines

In addition to standard qPCR primer design principles (replace U with T when converting an miRNA sequence to a DNA-based primer), the source provides these rules.

Forward Primer

1. Choose a forward primer of **12–18 bases**, leaving **4–8 bases** at the miRNA 3′ end for reverse-primer design.
2. Among the final five 3′ bases, include two to three A/T residues; among the final two 3′ bases, include one A/T residue.
3. If Tm is below 60°C, the source directs sequential addition of bases from **GACGC** at the 5′ end until Tm exceeds 60°C. The example provided adds three bases as **CAG–$N_{12–18}$,** with a longest illustrated form **CGCAG–$N_{12–18}$;** these sequence examples are reproduced as printed and require technical review before use.
4. If Tm exceeds 65°C, remove bases from the 5′ end until Tm is between 60°C and 65°C.

Reverse Primer

1. Select the appropriate miRNA 3′ sequence based on the forward-primer design step.
2. Add **15 T bases** to the reverse primer's 5′ end.
3. If Tm is below 60°C, the source directs adding bases from **GACCTGGAC** sequentially at the 5′ end until Tm exceeds 60°C. The illustrated forms are **CAG–T_{15}–$N_{4–8}$** and **CAGGTCCAG–T_{15}–$N_{4–8}$.** These are preserved as printed and require sequence-level technical confirmation.

Printed Example: hsa-miR-21

- miRNA sequence: **UAGCUUAUCAGACUGAUGUUG**
- Forward primer: **5′-GCAGTAGCTTATCAGACTGAT-3′**
- Reverse primer: **5′-GGTCCAGTTTTTTTTTTTTTTTCAAC-3′**

Technical review note: The sequence-design descriptions and examples in the original document contain apparent directional or sequence inconsistencies. This translation preserves the printed sequences and does not represent their experimental validation.

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Taq SYBR Green qPCR Premix (Universal)

Catalog No.: YZ-302003
Pack Size: 5 × 1 mL
Source Revision: HB260624
Product Information / Instructions for Use — English Translation

Product Description

Taq SYBR Green qPCR Premix (Universal) is a **2× ready-to-use mix for real-time quantitative PCR**. The blue formulation supports visual tracking during reaction setup. According to the manufacturer, the mix provides high sensitivity and specificity. The Taq DNA polymerase uses an antibody-mediated hot-start mechanism to help suppress nonspecific amplification caused by primer annealing during reaction preparation.

The formulation includes PCR amplification-enhancing factors and components intended to balance amplification of targets with different GC contents (**30–70%**), supporting a favorable linear relationship across a broad quantitative range as described in the source.

The product contains a special **ROX Passive Reference Dye** and is described in the source as suitable for various qPCR instruments without changing the ROX concentration. Primers and template are added during reaction setup. Verify instrument-specific performance when required.

Transport and Storage

Transport with ice packs. Store at **−20°C**, protected from light. **Shelf life: 18 months.** Avoid repeated freeze–thaw cycles. SYBR Green I is light-sensitive; protect the product from strong light during storage and reaction preparation.

Precautions

1. The manufacturer recommends the accompanying cDNA synthesis kit **YZ-302016-2** for removal of residual genomic DNA from RNA samples.
2. Upon thawing, flocculent or white precipitate may appear. Warm gently in the hand and invert carefully until fully dissolved; the source states this does not affect reagent performance.
3. Wear a lab coat and disposable gloves.
4. **For Research Use Only.**

Recommended 20 µL qPCR Reaction

Component	Volume	Final Concentration
Taq SYBR Green qPCR Premix	10 µL	1×
Forward primer (10 µM)	0.4 µL	0.2 µM
Reverse primer (10 µM)	0.4 µL	0.2 µM
DNA template	x µL	—
Sterile ultrapure water	To 20 µL	—

Notes:

1. Mix thoroughly before use, but avoid vigorous shaking that produces excessive bubbles.
2. Recommended primer concentration is **0.2 µM**, adjustable within **0.1–1.0 µM**.
3. Undiluted cDNA should not exceed **one-tenth of the reaction volume**.
4. A **5- to 10-fold** dilution of undiluted cDNA is recommended in the source; it identifies Ct values of approximately **20–30** as favorable for the intended analysis.

5. Prepare reactions in a clean laboratory workstation with nuclease-free pipette tips and PCR tubes. Filter tips are recommended to help reduce cross-contamination and aerosols.

qPCR Cycling Programs

A. Standard Cycling Program

Step	Temperature	Time	Cycles
Initial denaturation	95°C	2 min	1
Denaturation	95°C	10 s	40
Annealing/extension (collect fluorescence)	60°C	30 s	40
Melting curve	Instrument default	—	1

B. Fast Cycling Program

Step	Temperature	Time	Cycles
Initial denaturation	95°C	30 s	1
Denaturation	95°C	3 s	40
Annealing/extension (collect fluorescence)	60°C	20 s	40
Melting curve	Instrument default	—	1

The source states that the fast program is suitable for most targets; consider the standard program for targets with complex secondary structures. Adjust annealing conditions and timing according to primer and target length. Do not omit fluorescence acquisition.

Instrument acquisition time examples listed in the source: 20 s for ABI 7700/7900HT/7500 Fast; 31 s for ABI 7300; 32 s for ABI 7500. These examples should be checked against the actual instrument configuration.

Result Analysis

The source recommends at least **three biological replicates** for quantitative experiments. Examine amplification and melting curves after the reaction.

Amplification Curve

A typical amplification curve is sigmoidal (S-shaped).

- Ct **20–30**: the manual describes this as the most accurate range for quantitative analysis.
- Ct **<10**: dilute the template and repeat.
- Ct **30–35**: consider increasing template concentration or reaction volume, as indicated in the source.
- Ct **>35**: the manual says such results are not suitable for quantifying gene expression, but may be used qualitatively.

Important translation qualification: These Ct cutoffs are recommendations stated in this product manual, **not universal qPCR validity thresholds**. Quantitative suitability depends on assay-specific validation, efficiency, controls, and dynamic range.

Melting Curve

A single melting peak suggests favorable specificity for the tested reaction. Double or multiple peaks may indicate primer-dimer formation or nonspecific products; the source recommends evaluating the products by agarose gel electrophoresis when necessary.

For primer dimers, consider reducing primer concentration or redesigning primers. For nonspecific amplification, consider increasing annealing temperature or redesigning primers.

Primer Design Guidelines

1. Suggested primer length: approximately **25 bp**; ideal amplicon length approximately **150 bp**, selectable within **100–300 bp**.
2. Forward and reverse primer Tm difference should not exceed **2°C**; preferred Tm **60–65°C**.
3. Aim for evenly distributed bases, avoid runs of four identical bases, and use approximately **50% GC** content. A G or C at the final 3′ base is preferred.
4. Avoid complementary stretches of more than three bases within or between primers where possible.
5. Confirm specificity using **NCBI BLAST**, particularly 3′-end nonspecific complementarity.
6. Measure amplification efficiency after primer design; only primers with comparable amplification efficiencies should be used for quantitative comparisons.

Compatible Instrument Models Listed by the Manufacturer

Yuanzan: VQ-100, VQ-100A, VQ-100B, VQ-200.
ABI: 5700, 7000, 7300, 7700, 7900HT Fast, StepOne, StepOnePlus, 7500, 7500 Fast, ViiA 7, QuantStudio 3, 5, 6, 7, 12K Flex.
Stratagene: MX3000P, MX3005P, MX4000P.
Bio-Rad: CFX96, CFX384, iCycler iQ, iQ5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4.
Eppendorf: Mastercycler ep realplex, realplex 2 s.
Qiagen: Corbett Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000.
Roche Applied Science: LightCycler 480, LightCycler 2.0, LightCycler 96.
Thermo Scientific: PikoReal Cycler.
Cepheid: SmartCycler.
Illumina: Eco qPCR.

Compatibility note: This is the manufacturer's published list in the supplied manual; third-party manufacturer approval or independent validation is not implied.

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NovoStart® Fast SYBR qPCR SuperMix

Catalog No.: YZ-302010
Product Information / Instructions for Use — English Translation

1. Product Description

NovoStart® Fast SYBR qPCR SuperMix is a **2× ready-to-use mix** for fast real-time PCR using SYBR Green I intercalating fluorescence. The premixed format simplifies reaction setup. According to the source, a mutant Taq DNA polymerase and optimized reaction buffer help suppress nonspecific amplification at lower temperatures, enable faster PCR, and support reproducible quantification over a broad range.

2. Product Features (Manufacturer Statements)

1. **Fast:** The manual states that reaction time can be reduced by approximately **50%** compared with conventional qPCR.
2. **High specificity:** The source describes an antibody-modified **HotStart Super Taq DNA polymerase**, prepared by a new process to improve amplification specificity.
3. **Efficiency and sensitivity:** The source attributes the optimized real-time PCR formulation to **Novoprotein** and claims favorable amplification efficiency and sensitivity. The reference to Novoprotein is reproduced from the original document and should be checked before Yuanzan-branded publication.

Claim note: These are translated statements from the supplied instructions; no independent comparative dataset was included with this PDF.

3. Recommendations for Use

- Gently invert to mix and avoid bubbles; briefly centrifuge before use.
- Use new, clean tips and microtubes for reaction setup and aliquoting to reduce contamination.
- Recommended reaction volume: **20–50 µL**.
- Recommended target amplicon length: **under 200 bp**, where possible, to support amplification sensitivity/efficiency and reduce nonspecific amplification.

4. Quality Control

The manufacturer states that all components were tested and found to contain no residual endonuclease, exonuclease, or RNase activity.

5. Storage

Store at **−20°C**, protected from light. Avoid repeated freeze–thaw cycles.

6. Product Contents

Component	YZ-302010A	YZ-302010B
2× NovoStart® Fast SYBR qPCR SuperMix	5 × 1 mL	25 × 1 mL
RNase-free water	5 × 1 mL	23 × 1 mL
ROX Reference Dye I	200 µL	1 mL
ROX Reference Dye II	200 µL	1 mL

7. Typical 20 µL Reaction Setup

Component	Volume or Final Concentration

2× NovoStart® Fast SYBR qPCR SuperMix	10 µL
Forward primer	0.2–1.0 µM final
Reverse primer	0.2–1.0 µM final
Template	1–2 µL
ROX, if required by instrument	0.4 µL
RNase-free water	To 20 µL

8. Instrument Compatibility and ROX Selection

ROX I: ABI 5700/7000/7300/7700/7900/7900 HT/7900 HT Fast/StepOne/StepOnePlus, and other instruments.

ROX II: ABI 7500/7500 Fast/ViiA 7/QuantStudio 3/5/6 Flex/7 Flex/12K Flex; Stratagene MX4000/MX3500P/MX3000P; and other instruments.

No ROX required (examples listed): Bio-Rad CFX96/CFX384/iCycler iQ/iQ5/MyiQ/MiniOpticon/Opticon/Opticon 2/Chromo4; Eppendorf Mastercycler ep realplex/realplex 2s; Cepheid SmartCycler; Illumina Eco qPCR; Roche LightCycler 480/2.0/96; Thermo PikoReal; Qiagen Rotor-Gene Q/3000/6000.

Verify ROX requirements against the actual instrument user manual.

9. Cycling Programs

Step	Fast qPCR Program	Conventional qPCR Program
Initial denaturation	95°C, 30 s	95°C, 30 s
Number of cycles	35–45	35–45
Denaturation in each cycle	95°C, 5 s	95°C, 5 s
Annealing/extension in each cycle	60°C, 10–15 s	60°C, 30 s

10. Questions and Answers

Q1. Why is no Ct value reported?

Possible causes in the source:

1. **Incorrect fluorescence acquisition step:** For dye-based assays, fluorescence is generally collected during extension at 72°C; for probe-based assays, during or after annealing/extension, as stated in the source.
2. **Incorrect ROX selection or quantity:** Too much or too little ROX may alter corrected fluorescence and lead to missing Ct values. Choose ROX I or II according to the instrument and use the specified **1:50** ratio.
3. **Degraded primers or probes:** Check integrity by electrophoresis where appropriate.
4. **Insufficient template:** Start with the highest concentration in a serial dilution series when sample concentration is unknown.
5. **Degraded template:** Avoid impurities and repeated freezing/thawing; prepare fresh template if necessary.

Q2. Why is Ct late (Ct > 38)?

- Low amplification efficiency or insufficiently optimized cycling; redesign primers/probes, adjust annealing conditions, or consider a three-step program.
- Degraded reagents or insufficient input; replace reagents or increase template input and repeat.
- Excessive product length; use an **80–150 bp** target where feasible.
- Insufficient hot-start activation; the FAQ recommends a hot-start duration of at least **1 minute**.

Q3. Why is the amplification curve discontinuous or sawtooth-shaped?

- Inappropriate ROX selection or volume can affect corrected fluorescence.

- Wrong acquisition channel or using ROX data processing when no ROX was added.
- Excessively concentrated or degraded template, or degraded fluorescent dye.
- Bubbles within the reaction tube; mix and centrifuge to eliminate bubbles.
- Insufficient instrument acquisition or extension time; the source suggests **40–60 seconds** when needed.

Q4. Why is the standard curve poorly linear?

- Pipetting inaccuracies preventing the expected dilution gradient.
- Degraded standards caused by repeated freeze–thaw cycles.
- Poor primer/probe design.
- Template inhibitors or excessive template concentration; replace or dilute the template.

Q5. Why does the negative control show a signal?

- Suboptimal primers (primer dimers or hairpins).
- Inappropriate primer concentration or forward/reverse ratio.
- Genomic DNA contamination during RNA preparation.
- Reaction contamination; replace mix, water or primers and prevent carryover.

Q6. Why is there more than one major melting peak?

- Excessive primer concentration or suboptimal design.
- Reagent contamination.
- Nonspecific amplification caused by unsuitable conditions; improve primer design or increase annealing temperature.

Q7. How should different curves on different instruments be evaluated?

The source proposes considering **amplification efficiency, sensitivity, and specificity** and states that efficiencies of **90–110%** are acceptable when amplification is specific.

Q8. Why is melting temperature (Tm) not close to 80°C?

The source states that an amplicon of about 100 bp may show a peak near 80°C and suggests low Tm may reflect template degradation. **This is the source's troubleshooting statement; melting temperature is sequence- and assay-dependent.**

Q9. Can the melting-curve step be omitted?

The source allows omission when amplification performance and specificity are already established but recommends including a melting curve and adjusting settings for the instrument.

Q10. Why is efficiency low in a 5 µL reaction?

Small volumes are more sensitive to changes in primer/template concentration and divalent-metal-ion levels, which may reduce amplification efficiency.

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First-Strand cDNA Synthesis SuperMix for qPCR (gDNA digester plus)

Catalog No.: YZ-302016-2
Pack Size: 100 reactions
Source Revision: HB260624
Product Information / Instructions for Use — English Translation

Product Description

First-Strand cDNA Synthesis SuperMix for qPCR (gDNA digester plus) is a reverse transcription kit that includes a genomic DNA removal step. It is designed for **two-step RT-qPCR**.

The kit contains **5× gDNA digester Mix** and **4× First-Strand cDNA Synthesis SuperMix**. The 5× gDNA digester Mix removes residual genomic DNA from RNA preparations. The 4× First-Strand cDNA Synthesis SuperMix contains the components required for reverse transcription: buffer, dNTPs, reverse transcriptase, RNase inhibitor, and a random primer/oligo(dT)$_{18}$ primer mixture. Add RNA template and RNase-free water as described in the procedure. The reverse transcription step also terminates gDNA digester activity, as described in the source.

Product Contents

Component	Volume
RNase-free H_2O	2 × 1 mL
5× gDNA digester Mix	320 µL
4× First-Strand cDNA Synthesis SuperMix	500 µL

Transport and Storage

Ship with ice packs. Store at **−20°C**. **Shelf life: 18 months.**

Precautions

1. The 5× gDNA digester Mix and 4× First-Strand cDNA Synthesis SuperMix contain high glycerol concentrations. Briefly centrifuge to collect liquid at the bottom of the tube, then gently pipette to mix thoroughly before dispensing.
2. When using a large RNA template volume (for example, over 2 µL), ensure that the RNA is dissolved in **water rather than TE buffer**, because TE can inhibit both gDNA removal and reverse transcription.
3. Prepare reactions on ice and protect against RNase contamination.
4. Wear a laboratory coat and disposable gloves.
5. **For Research Use Only.**

First-Strand cDNA Synthesis Procedures

A. When Residual Genomic DNA Removal Is Required

Step 1: Removal of Residual Genomic DNA

Prepare the following mixture in an RNase-free microcentrifuge tube and gently mix by pipetting. Incubate at **42°C for 2 minutes**.

Component	Amount
RNase-free H_2O	To 15 µL
5× gDNA digester Mix	3 µL
Total RNA	10 pg–5 µg

OR mRNA	10 pg–500 ng

For a 20 µL reverse transcription reaction, the source recommends **no more than 1 µg total RNA** under routine conditions. Up to 5 µg can be added for low-abundance targets, but excessive RNA may exceed the quantitative range in subsequent qPCR.

Step 2: Prepare the Reverse Transcription Reaction (20 µL)

Add the 4× First-Strand cDNA Synthesis SuperMix directly to the Step 1 reaction. Mix gently by pipetting.

Component	Volume
Reaction mixture from Step 1	15 µL
4× First-Strand cDNA Synthesis SuperMix	5 µL

Step 3: Select a Reverse Transcription Program

Program A — Standard

Temperature	Time
25°C	5 min
55°C	15 min
85°C	5 min

The source recommends **55°C** for reverse transcription. For high-GC or structurally complex RNA templates, the temperature may be increased to **60°C**.

Program B — Fast (Low/Moderate GC or Simple Templates)

Temperature	Time
55°C	15 min
85°C	5 s

Reverse transcription products may be used immediately for qPCR or stored at **−20°C** for a short period. For long-term storage, the source recommends aliquoting and keeping at **−80°C** to minimize repeated freeze–thaw cycles.

B. When Residual Genomic DNA Removal Is Not Required

Step 1: Prepare the Reverse Transcription Reaction (20 µL)

Component	Amount
RNase-free H_2O	To 20 µL
4× First-Strand cDNA Synthesis SuperMix	5 µL
Total RNA	1 ng–5 µg
OR mRNA	1 ng–500 ng

Step 2: Reverse Transcription Program

Use the standard or fast reverse transcription program listed above.

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