# AccelerRT® 5' RACE Kit User Manual

For 5' RACE reverse transcription and amplification

Cat. No: PC060 (10 reactions)

## User Manual

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# AccelerRT® 5' RACE Kit



## I. Description

RACE (Rapid Amplification of cDNA Ends) is a technique that enables rapid amplification of the unknown sequences at the end of the cDNA, using known partial sequences as an anchor. The AccelerRT® 5′ RACE Kit enables efficient reverse transcription of cDNA from 10 ng to 1 μg of total RNA, followed by rapid amplification of the 5′ ends using the synthesized cDNA as the template.

In 5′ RACE, upon reaching the 5′ end of the RNA template, a specific adapter sequence is annealed and extended to the 3′ end of the cDNA by the terminal deoxynucleotidyl transferase (TdT) activity of the Template Switching Reverse Transcriptase, eliminating the need for separate adapter ligation steps. The PCR amplification system utilizing the 2× PCR Amplification Mix enables amplification of long fragments and GC-rich regions, while maintaining high specificity to minimize smearing in RACE experiments.

This kit provides all pre-optimized reagents for 5′ RACE. Altering component concentrations or substituting with equivalent products is not recommended and may yield suboptimal results. Prior validation is required if substitution is necessary.

**Advantages**

- Streamlined procedure with no adapter ligation steps required
- 5′ RACE RT Mix delivers robust reverse transcription and template-switching activity for efficient 5′ RACE fragment generation
- 2× PCR Amplification Mix offers high amplification efficiency and fidelity to obtain accurate target fragments
- Compatible with 10 ng–1 μg of total RNA or poly(A)+ RNA as input template

## II. Contents and Storage

Cat. No: PC060 (10 reactions)

| Cat. No. | Contents | Part No. | Quantity | Store |
|---|---|---|---|---|
| PC060 (10 reactions) | 5' RACE TS Oligo (20 μM) | PC060-01 | 10μl | -80℃ |
| | RNase Inhibitor (25 U/μl) | PC060-02 | 10μl | -20℃ (stable for at least 12 months) |
| | 5' RACE RT Primer | PC060-03 | 10μl | |
| | 5× RACE RT Buffer | PC060-04 | 40μl | |
| | 5' RACE RT Mix | PC060-05 | 10μl | |
| | 5' Random Primer(20 μM) | PC060-06 | 10μl | |
| | 2× PCR Amplification Mix | PC060-07 | 650μl | |
| | 10× Universal Primer Mix | PC060-08 | 100μl | |
| | Short PCR Primer (10 μM) | PC060-09 | 50μl | |
| | dNTP Mix (25mM) | PC060-10 | 10μl | |
| | Dilution Buffer | PC060-11 | 1ml | |
| | ddH2O (RNase/DNase free) | QP006-07 | 1ml | |

## III. Preparation

### ■ Contamination Prevention

1) The detection sensitivity of the kit is high, and it is necessary to avoid experimental cross-contamination. It is recommended to separate the experimental area of cDNA synthesis from that of PCR reaction, and clean the experimental area regularly.

2) In order to prevent the degradation of RNA, solution reagents, utensils, gun heads, centrifugal tubes, etc. used in the experimental reaction process should be treated with DEPC water as far as possible, and then used after autoclaving. Wear disposable gloves and avoid talking during the experiment.

3) To avoid cross-contamination of experimental samples, it is recommended to use a nuclease free gun head with a filter element, absorb different components to replace the gun head, and wipe the pipette and desktop with 75% ethanol after the experiment.

4) For the first experiment, to avoid false positives caused by environmental pollution, it is recommended to design a negative control.

### ■ Sample Requirements

RNA samples

For best results, use high quality Poly(A) RNA and total RNA samples with high integrity and purity. Make sure the RNA does not contain contaminants such as residual proteins, organic solvents, and salts that can degrade the RNA or reduce enzyme activity and sensitivity. Before the experiment, the Agilent RNA 6000 Pico Kit could be used to evaluate RNA integrity, and RNAs with RIN≥7 were recommended. If needed, the PC061AccelerRT® 5 'RACE Control Kit can be purchased for testing.

■ **Required Materials not Included**

1) 5 'GSP: PCR 5' gene-specific primer

2) Gel Extraction/Purification Kits: QIAGEN QIAquick Gel Extraction Kit (Cat.No. 28704), Omega E.Z.N.A® Cycle-Pure Kit (Cat.No. D6492), or other equivalent product.

3) Cloning Kits: SmartJoin™ Blunt-End PCR Cloning Kit (Cat.No. IC007) or other equivalent product.

4) Transformation Reagents: DH5α Competent Cells (Cat.No. CC001) or other equivalent product.

5) Other Materials: 0.2mL PCR tube, low adsorption 1.5mL EP tube, $ddH_2O$.

6) RACE Amplification Reagents: 2× UltraHiPF® PCR Mix (Cat.No. PC033) or other equivalent product.

■ **Primer Design Guidelines:**

1) Length: 23-28 nucleotides recommended, should not exceed 30 nt.

2) GC Content: Maintain between 50%-70%.

3) Tm Value: Ensure Tm ≥65°C for specific annealing. For Tm >70°C, use touchdown PCR. If Tm <65°C, adjust annealing temperature.

4) Long Transcripts: Design primers closer to the 5' end, keep PCR products under 3 kb.

5) GSP Requirement: GSP must not complement the 3' end of the 10× Universal Primer Mix.

Long primer:

5’-CATACATTCACATACGTAGGGCAAGCAGTGGTATCAACGCAGAGT-3’

Short primer:

5’-CATACATTCACATACGTAGGGC-3’

6) Nested GSP (NGSP) Design:

Follow same parameters for length, GC content, and Tm as GSP.

Position NGSP near the 3' end of the GSP binding site, ensuring no 5' end overlap with GSP.

# IV. Procedures

## Template Switching Mechanism

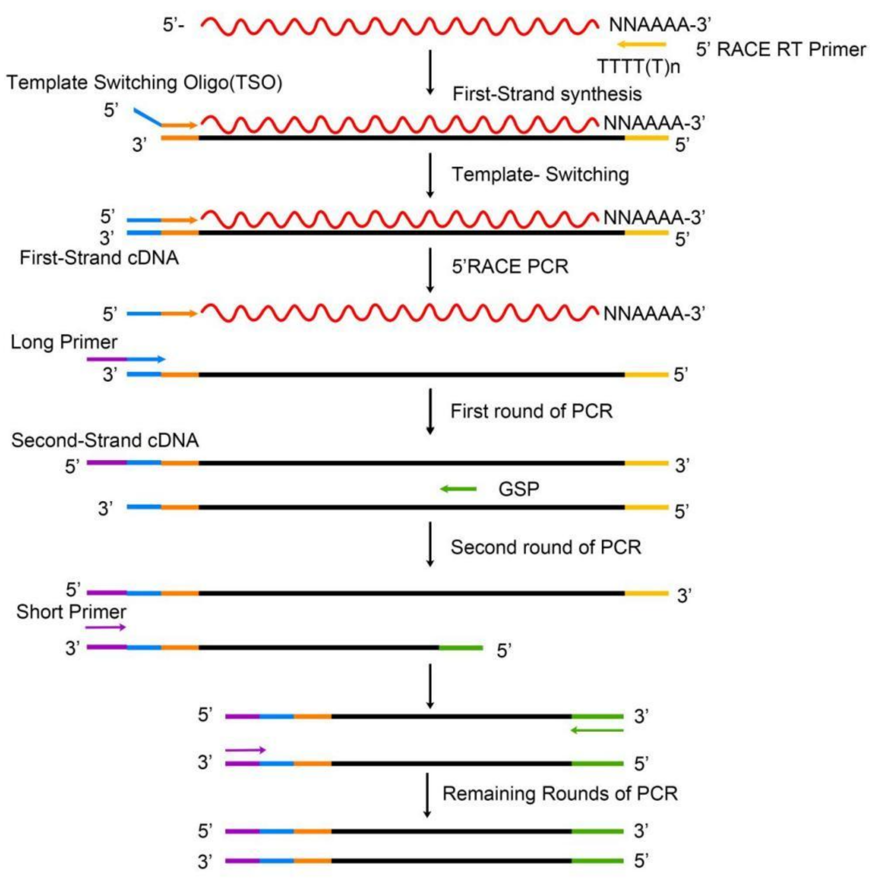


Figure 1.Schematic Diagram of 5′ RACE Amplification Principle.

## Experimental Procedure

- **Reverse Transcription Reagent Preparation**

1. Thaw all the reagents. Mix reagents well by gently inverting the tubes. Spin down briefly and keep on ice.
2. Prepare **Mix1** according to the table below, adding the reagent components required for each reaction to the PCR tube.

| Reagent | Volume |
|---|---|
| RNA | 10ng~1μg |
| 5' RACE RT Primer*[a] | 1μl |
| dNTP Mix (25mM) | 1μl |
| ddH2O (RNase/DNase free) | add to 10 μl |

*a: If the transcript lacks a poly(A) tail, substitute the 5′ RACE RT Primer with the 5′ Random Primer.

3. Mix the reaction solution well. Spin down briefly.

4. Incubate at 72°C for 3 minutes, then immediately place on ice.
5. Prepare **Mix2** according to the table below, adding the reagent components required for each reaction to the PCR tube.

| Reagent | Volume |
|---|---|
| 5× RACE RT Buffer | 4 μl |
| RNase Inhibitor (25 U/μl) | 1 μl |
| 5' RACE RT Mix | 1 μl |
| 5' RACE TS Oligo(20 μM) | 1 μl |
| $ddH_2O$ (RNase/DNase free) | 3 μl |
| Total | 10 μl |

6. Mix the solution well. Spin down briefly.

- **Reverse Rranscriptional Reaction**

1. Add **Mix2** to **Mix1**, mix the solution well and spin down briefly.
2. Incubate according to the following procedure:

| Temperature | Time |
|---|---|
| 42℃ | 90 min |
| 85℃ | 5 min |
| 4℃ | ∞ |

◎ Stopping point: Samples can be stored at -20 ° C.

- **PCR reaction**

1. Thaw all the reagents. Mix reagents well by gently inverting the tubes. Spin down briefly and keep on ice.
2. cDNA Dilution Reference:

| Starting Template (RNA) | cDNA volume | Dilution Buffer*b |
|---|---|---|
| ≤200ng RNA | 10 μl | 10 μl |
| >200ng RNA | 10 μl | 40 μl |
| Poly A RNA | 10 μl | 40 μl |

*b: The dilution factor should be optimized based on the starting RNA amount and abundance of the target gene.

3. Add the reagent components required for each reaction to the PCR tube on the ice according to the table below.

| Reagent | 5'RACE | UPM single primer control (optional) | GPS single primer control (optional) |
|---|---|---|---|
| 2× PCR Amplification Mix | 12.5 μl | 12.5 μl | 12.5 μl |
| 10×Universal Primer Mix | 2.5 μl | 2.5 μl | - |
| 5' GSP (10 μM) | 1 μl | - | 1 μl |
| cDNA | 2 μl | 2 μl | 2 μl |
| $ddH_2O$ (RNase/DNase free) | Up to 25 μl | Up to 25 μl | Up to 25 μl |

4. Mix the reaction solution well. Spin down briefly. Make sure there are no bubbles in the reaction solution and that it is placed at the bottom of the PCR tube or plate.
5. Set the PCR procedure according to the following table (take the reaction procedure recommended by Takara PCR instrument as an example):

**Program 1:** The Tm value of GSP is 60 to 70℃.

| Temperature | Time | cycle |
|---|---|---|
| 98℃ | 2 min | 1 |
| 98℃ | 10 sec | 20~25*d |
| 63~68℃*e | 10 sec | |
| 72℃ | 3min*c | |
| 72℃ | 5 min | 1 |
| 4℃ ∞ | | |

**Program 2:** The Tm value of GSP is ≥70℃ (Touchdown PCR).

| Temperature | Time | cycle |
|---|---|---|
| 98℃ | 2 min | 1 |
| 98℃ | 10 sec | 5 |
| 72℃ | 3min*c | |
| 98℃ | 10 sec | 5 |
| 70℃ | 10 sec | |
| 72℃ | 3 min*c | |
| 98℃ | 10 sec | 20~25*d |
| 68℃ | 10 sec | |
| 72℃ | 3 min*c | |
| 72℃ | 5 min | 1 |
| 4℃ ∞ | | |

*c: For amplification of 3 kb fragments, set the extension time to 3 minutes. If the fragment exceeds 3 kb, add 1 minute per additional kb.

*d: If target band relatively is weak, appropriately increase the final amplification cycle number.

*e: If the specific primer Tm is below 65°C, adjust the 68°C annealing temperature in Program 1 accordingly. The annealing temperature must not exceed Tm + 3°C. If using the PC061 AccelerRT® 5' RACE Control Kit, set the Tm to 63°C in Program 1.

- **Nested PCR (Optional)**

When no target band or faint bands are observed in the first-round amplification, perform nested PCR using NGSP to obtain higher specificity and yield.

1. Thaw all the reagents. Mix reagents well by gently inverting the tubes. Spin down briefly and keep on ice. Transfer 5 μl of the first-round PCR product to the 1.5 mL EP tube and add 245 μl $ddH_2O$ (if no target band is detected, optimize the dilution factor). Mix the reaction solution well.

2. Add the reagent components required for each reaction to the PCR tube on the ice according to the table below.

| Reagent | 5'RACE | Short PCR Primer single primer control (optional) | NGSP single primer control (optional) |
|---|---|---|---|
| 2× PCR Amplification Mix | 12.5 μl | 12.5 μl | 12.5 μl |
| Short PCR Primer (10 μM) | 1 μl | 1 μl | - |
| 5' GSP (10 μM) | 1 μl | - | 1 μl |
| 1st PCR diluted product | 2 μl | 2 μl | 2 μl |
| $ddH_2O$ (RNase/DNase free) | Up to 25 μl | Up to 25 μl | Up to 25 μl |

3. The amplification procedure refers to the first-round PCR procedure.

- **RACE PCR Product Purification**

Purified amplification products should be validated by cloning and sequencing. If a single band is observed, purify using the Omega E.Z.N.A® Cycle-Pure Kit (Cat.No. D6492). For multiple bands, use the QIAGEN QIAquick Gel Extraction Kit (Cat.No. 28704).

- **Cloning & Transformation**

1. The 2× PCR amplification generates blunt-end products, compatible with our SmartJoin™ Blunt-End PCR Cloning Kit (Cat.No. IC007) or equivalent. Refer to the product manual for detailed protocols.
2. Transformation: Standard E. coli competent cells (e.g.,GeneCopoeia DH5α Competent Cells, Cat.No.CC001) are suitable. Follow the manufacturer's instructions.
3. Positive Clone Screening: Pick single colonies for colony PCR screening using 2× UltraHiPF® PCR Mix (Cat.No. PC033). Screen 8–10 clones to maximize full 5′-end sequence coverage.
4. Sequencing Analysis: Verify insertion of the target fragment by Sanger sequencing. Use vector primers included in the SmartJoin™ Blunt-End PCR Cloning Kit (Cat.No. IC007) or custom-designed primers.

## V. FAQ

| Question | Possible cause | Recommended action |
| --- | --- | --- |
| No Amplification or Smearing | No target transcript in RNA. | Verify RNA quality by designing PCR primers for a known region of the target gene. |
| | RNA degradation or contamination. | Assess RNA purity and integrity post-extraction (e.g., agarose gel electrophoresis or Agilent analysis). |
| | Low target gene abundance. | Increase RNA input (up to 4 μg).<br>Increase cDNA input volume (≤1/10 of PCR reaction volume).<br>Raise GSP input (10 μM, ≤1/10 of PCR volume).<br>Design nested GSP for multi-round PCR (≤3 rounds).<br>Extend PCR cycles (≤40 cycles; ≤50 for touchdown PCR). |
| | GSP Tm too low. | Design GSP with Tm ≥65°C. Use touchdown PCR if Tm >70°C; if Tm <65°C, reduce annealing temperature (≤ Tm + 3°C). |
| | Unsuitable GSP design. | Redesign and test multiple new GSP candidates. |
| | RACE product from non-poly(A) RNA. | Use kit-provided 5′ Random Primer for cDNA synthesis, followed by nested GSP amplification. |
| | PCR amplicon too long. | Adjust extension time (±1 min/kb); design GSP closer to the 5′ end. |
| Multiple Bands | Multiple transcript variants. | Analyze splice variants and redesign specific GSP. |
| | Non-specific amplification. | Redesign GSP (Tm ≥65°C), validate specificity via BLAST. |
| No Positive Clones | Low product concentration. | Scale up PCR reactions, pool/purify products, and increase insert amount in cloning. |

## VI. Limited Use License and Warranty

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The following terms and conditions apply to the use of AccelerRT® 5' RACE Kit (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged, or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

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GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia’s liability is expressly limited to replacement of Product, or a refund limited to the actual purchase price. GeneCopoeia’s liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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