

# ExactSTART™ Eukaryotic mRNA 5' - & 3' - RACE Kit

Cat. No. ES80910 – 10 Reactions

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## 1. Introduction

The ExactSTART Platform of Transcriptome Discovery and Analysis Tools

The ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit is one component of the ExactSTART Platform of transcriptome discovery and analysis tools from Epicentre. The ExactSTART (START: Selective Tagging and Amplification of RNA Transcripts) Platform kits combine the advantages of T4 RNA Ligase-mediated RNA tagging with a select group of RNA modifying enzymes that have strict enzymatic specificity. When used in a precise order, these enzymes enable the researcher to selectively “tag” the 5' and 3' ends of a specific class of RNA, such as 5'-capped RNAs (e.g., eukaryotic mRNAs and eukaryotic viral RNAs), 5'-triphosphorylated RNAs (e.g., some noncoding RNAs), 5'-monophosphorylated RNAs (e.g., miRNAs), as well as other identified RNAs or RNAs whose functions are not yet identified. By incorporating 5' and 3' tags with unique functional sequences, the selected class of transcripts can be amplified for a specific downstream application such as next-gen DNA sequencing, RACE, cloning, RT-PCR, microarray, and other gene discovery and analysis methods.

**Important!** *The components of the ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit are formulated specifically and solely for use with this kit. DO NOT use any component of this kit with any other ExactSTART Kit. DO NOT use any component of another ExactSTART Kit.*

**Storage:** Upon receipt of this kit, remove the tubes containing the HeLa Total RNA Control and 5'-RACE Acceptor Oligo and store them at -70°C. Store the remainder of the kit at -20°C.

### Additional Required Reagents and Equipment

Thermostable DNA polymerase (preferably with proofreading activity)

Thermocycler or heating block (multiple temperatures needed)

Thin-walled PCR tubes

TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA), RNase-free

80% Ethanol (cold)

1:1 phenol (buffered):chloroform

3 M Sodium acetate

24:1 chloroform:isoamyl alcohol

Isopropyl alcohol (isopropanol)

## 2. Kit Contents

The kit components are supplied in tubes with colored caps for easier identification.

Component Name	10 Reactions	Cap Color
APex Heat-Labile Alkaline Phosphatase	60 µl	Green
APex Reaction Buffer	120 µl	
Glycogen	15 µl	
Tobacco Acid Pyrophosphatase (TAP)	15 µl	Red
TAP Buffer	15 µl	
RiboGuard RNase Inhibitor	10 µl	Yellow
T4 RNA Ligase	15 µl	
RNA Ligase Buffer	30 µl	
TAP STOP Buffer	15 µl	
5'-RACE Acceptor Oligo*	15 µl	
2 mM ATP Solution	15 µl	
MMLV Reverse Transcriptase	15 µl	Blue
MMLV Reverse Transcriptase Buffer	30 µl	
cDNA Synthesis Primer	15 µl	
dNTP PreMix	30 µl	
RNase Solution	15 µl	
FailSafe PCR PreMix E	2 x 1 ml	
PCR Primer 1	350 µl	
PCR Primer 2	350 µl	
Nuclease-Free Water	2 x 1.5 ml	Clear
HeLa Total RNA Control (200 ng/µl)*	15 µl	
β-Actin Forward Control Primer	10 µl	
β-Actin Reverse Control Primer	10 µl	

## 3. Performance Specifications and Quality Control

The ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit is function-tested in 5'- & 3'-RACE control reactions. The 5'-RACE control reaction yields a ~400 bp product and the 3'-RACE control reaction yields a 250-300 bp product.

All components of the ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit are free of detectable RNase and DNase activities as judged by agarose gel electrophoresis following overdigestion assays, with the exception of the inherent nucleolytic functions of the MMLV-RT and ExactSTART RNase Mix components.

## 4. Features and Benefits of the ExactSTART Eukaryotic mRNA 5′- & 3′-RACE Kit

1. Enables simultaneous 5′ and 3′ RACE of eukaryotic RNA with both 5′-capped and 3′-polyadenylated ends.
2. Provides extremely strong selection for full-length 5′-capped and 3′-polyadenylated RNAs in the total RNA Preparation.
3. Preserves the extreme 5′ end of the mRNA molecule, allowing for the accurate mapping of transcriptional start sites.
4. Flexible: alternative RACE strategies can be employed.
5. Fast and easy process can be completed in less than 1 day with less “hands-on” time than other kits.
6. Enables discovery of new, alternate transcription start sites and alternative polyadenylation sites.
7. Captures the true transcriptional start site (5′ end), and polyadenylation site (3′ end) without the sequence ambiguity often created by other systems.

## 5. Kit Overview

The basic workflow of the kit is outlined in Fig. 1. The ExactSTART Eukaryotic mRNA 5′- & 3′-RACE Kit facilitates the precise identification of the 5′ and 3′ ends of eukaryotic mRNA that have: 5′-capped / 3′-polyadenylated ends (such as, but not limited to, 5′ m7GpppN-----AAAAA... 3′).

**The kit process uses three RNA modifying enzymes:** APex™ Heat-Labile Alkaline Phosphatase, Tobacco Acid Pyrophosphatase (TAP), and T4 RNA Ligase, and an RNA ligation-mediated adaptor “tagging” process to add PCR priming sites to both the 5′ and the 3′ ends of RNAs that have both a 5′-capped and 3′-polyadenylated end, such as eukaryotic mRNAs. RNAs that lack either or both of these features are generally excluded from the population of RACE-amplified DNA that is produced by the kit. However, alternative RACE strategies can be employed.

- A) Alkaline Phosphatase Treatment.** The total RNA sample is treated with APex Heat-Labile Alkaline Phosphatase which converts RNA with a 5′ monophosphate or a 5′ triphosphate to RNA with 5′-hydroxyl ends to ensure that they will not participate in the ExactSTART RACE amplification process. 5′-capped RNAs, such as eukaryotic mRNA, are not affected by the APex Phosphatase treatment.
- B) Tobacco Acid Pyrophosphatase Treatment.** The phosphatase-treated sample is treated with TAP, which removes the cap structure that is present at the 5′ end of eukaryotic mRNAs, yielding mRNA with a 5′ monophosphate. RNA with a 5′ monophosphate is required for the 5′-ligation-tagging step (Part C).
- C) 5′-Ligation-Tagging.** The 5′-RACE Acceptor Oligo is ligated to the 5′ end of the TAP-treated RNAs using T4 RNA Ligase. The 5′-RACE Acceptor Oligo contains a PCR priming site for the subsequent amplification step. Only those RNAs with a 5′ monophosphate will participate in the ligation reaction.

- D) **cDNA Synthesis.** 3'-polyadenylated RNAs are reverse transcribed into cDNA. cDNA synthesis is primed by the cDNA Synthesis Primer, which consists of an oligo(dT) sequence with a PCR priming site sequence (different from the tagging sequence of the 5'-RACE Acceptor Oligo described above) at its 5' end. Only those RNAs with a 3'-polyadenylated tail will be converted to cDNA.
- E) **Second-Strand cDNA Synthesis and PCR Amplification.** The ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit process uses PCR amplification to generate the second strand of the cDNA, and then to amplify the resulting double-stranded cDNA (dsDNA). The PCR Primer 1 and PCR Primer 2 anneal to the PCR priming sites present in the 5'-RACE Acceptor Oligo and the cDNA Synthesis Primer sequences. Only those cDNAs from Part D that contain both the 5'-RACE Acceptor Oligo sequence and the cDNA Synthesis Primer sequence will be converted to amplified dsDNA.

## 6. Alternative Strategies for Using the ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit

- A. Transcript-Specific RACE. The ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit, as provided, yields a RACE-amplified pool of double-stranded cDNA produced from all RNA containing both a 5'-capped and a 3'-polyadenylated end. However, amplification of a specific RNA transcript of interest can be performed if a portion of the sequence of the transcript is known. To perform transcript-specific RACE using this kit:
- 1) Perform Part A-C as described.
  - 2) In Part D, substitute the transcript-specific primer (provided by the user) for the cDNA Synthesis Primer and continue with the cDNA synthesis reaction as described.
  - 3) In Part E, substitute the transcript-specific primer for the PCR Primer 2. It may be necessary to optimize the PCR when using a custom primer.
- B. RACE-amplification of 5'-capped/3'-polyadenylated RNAs, 5'-monophosphorylated/3'-polyadenylated RNAs, and 5'-triphosphorylated/3'-polyadenylated RNAs. By eliminating the APex Phosphatase treatment (Part A), all RNAs containing either a 5' cap, 5' monophosphate, or 5' triphosphate and a 3'-polyadenylated end can be 5' tagged and amplified. The TAP treatment of Part B will convert the 5'-capped and 5'-triphosphorylated RNAs to 5'-monophosphorylated RNAs that are required for ligation of the 5'-RACE Acceptor Oligo (Part D).

## 7. Notes and Considerations

1. **Thin-Walled PCR Tubes and Thermocycler:** Researchers are strongly encouraged to use a thermocycler and thin-walled PCR tubes for all of the steps and incubations in this protocol, even those not involving PCR. If a thermocycler is not readily available for all the steps, standard heat blocks can be used, but care should be taken to ensure the temperatures are accurate and excessive sample evaporation does not occur.
2. **Input RNA Considerations:** The quality of the input RNA is critical for both the yield and the representation of the RACE-amplified cDNA produced. The cDNA synthesis process used in the ExactSTART 5'- & 3'-RACE Kit is designed to specifically

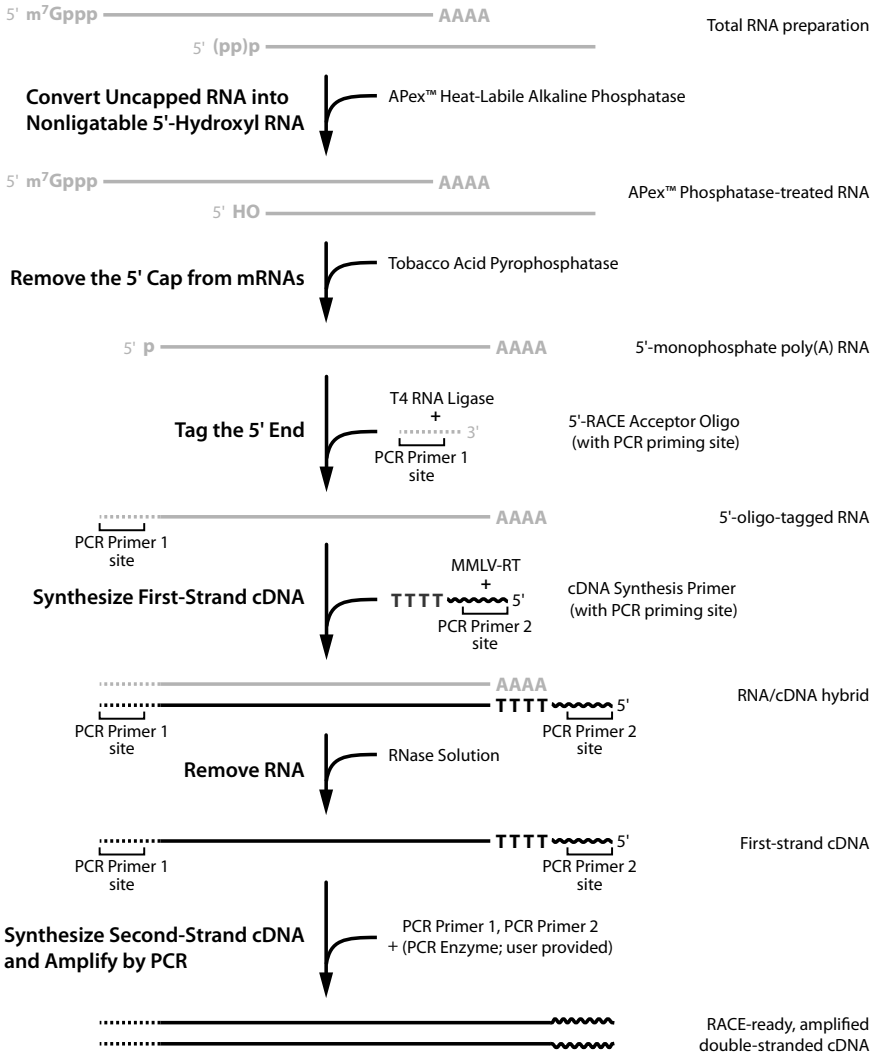


Figure 1. The ExactSTART™ Eukaryotic mRNA 5'- & 3'-RACE Kit Procedure.

exclude partially degraded mRNAs that do not have both a 5'-cap structure and a 3'-polyadenylated end. Therefore, the starting RNA material needs to be of extremely high quality. Epicentre offers a broad range of RNA purification kits that are able to purify intact RNA from a wide variety of starting materials.

The standard ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit reaction uses 1-10 µg of total RNA. The RNA should be dissolved in Nuclease-Free Water or RNase-free TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

- Standard RNA "Good Practices":** The use of standard laboratory "good practices" is critical when working with RNA due to the ubiquitous presence of RNases on most laboratory surfaces and human skin. The use of gloves, aerosol barrier pipette tips, and certified RNase-free sample tubes is strongly encouraged. It is also advisable to create an RNase-free area in which work is performed. Surface cleaners that specifically remove and inactivate RNases are available from many vendors.
- PCR Amplification:** The ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit requires the use of PCR amplification. The kit includes the FailSafe™ PCR PreMix E which contains buffer, dNTPs, and betaine<sup>+</sup>, a PCR enhancer. The user must provide a thermostable DNA polymerase, preferably with proofreading activity.
- Alternative PCR enzymes.** This kit is optimized for use with Epicentre's FailSafe™ PCR Enzyme. However, alternative proof-reading PCR enzymes can be used. If using an alternative PCR enzyme:
  - Use the reaction buffer that is provided with the alternative PCR enzyme. **Do Not** use the FailSafe™ PreMix E that is provided in this kit.
  - If the alternative PCR enzyme's buffer does not contain dNTPs, then add dNTPs to a final concentration of 0.2mM each dNTP.

## 8. ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit Procedure

**Important!** *The components of the ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit are formulated specifically and solely for use with this kit. DO NOT use any component of this kit with any other ExactSTART Kit. DO NOT use any component of another ExactSTART Kit with this kit.*

Please review the Procedure and read all of the Notes and Considerations before attempting the procedure. If you have any questions please contact our Technical Support Scientists.

### A. Alkaline Phosphatase Treatment

This procedure will remove the 5'-phosphate group(s) from 5'- mono-, di-, and triphosphorylated RNAs in the total RNA sample. RNAs containing a 5'-cap structure (for example m7GpppGpN,...) will not be affected by the APex Phosphatase treatment.

The protocol below recommends clean up with phenol:chloroform extraction before the RNA is used in Part B. Although APex Phosphatase is heat-labile, heat-inactivation of the alkaline phosphatase should be avoided as it could cause degradation of the RNA sample due to the Mg<sup>2+</sup> present in the APex Reaction Buffer.

## Required in Part A

Component Name	Tube Label	Cap Color
APex Heat-Labile Alkaline Phosphatase	APex Phosphatase	Green
APex Reaction Buffer	APex Buffer	
Glycogen	Glycogen	
Nuclease-Free Water	Nuclease-Free Water	Clear

**Additional Required Reagents (provided by the user)**

1:1 Phenol (buffered):chloroform

Chloroform:Isoamyl alcohol (24:1)

80% Ethanol (cold)

3 M Sodium acetate

Isopropyl alcohol (isopropanol)

## 1. Assemble the following reaction:

x	μl	Nuclease-Free Water
10	μl	APex Reaction Buffer
x	μl	Total RNA sample (1-10 μg)
5	μl	APex Heat-Labile Alkaline Phosphatase
<hr/>		
100	μl	Total reaction volume

- Incubate the reaction at 37°C for 15 minutes.
- Clean up the sample by phenol:chloroform extraction or another method. If performing phenol: chloroform extraction, follow Part A, Steps 4-12. If purifying by another method, such as a spin-column, follow the manufacturer's recommended procedure; then, adjust the volume/concentration of the eluted RNA to 1-10 μg RNA in 7.5 μl.
- For phenol:chloroform extraction: Add 1 μl of Glycogen to the tube. Then, add 100 μl of 1:1 phenol (buffered):chloroform and vortex vigorously. Centrifuge at 10,000 x g for ~1 minute to separate the two phases. Collect and save the aqueous phase (upper phase) to a new sterile tube.
- Add 100 μl of Nuclease-Free Water to the phenol:chloroform phase and vortex vigorously. Centrifuge at 10,000 x g for about 1 minute to separate the two phases. Collect the aqueous phase (upper phase) and combine it with the aqueous phase collected previously in Part A, Step 4.
- Add 200 μl of chloroform:isoamyl alcohol (24:1) to the combined aqueous phases (~200 μl) and vortex vigorously. Centrifuge at 10,000 x g for about 1 minute to separate the two phases. Collect and save the aqueous phase (upper phase) to a new sterile tube. The aqueous phase contains the RNA.
- Add 20 μl of 3 M sodium acetate and 100 μl of isopropyl alcohol (isopropanol) to the tube containing the aqueous solution and vortex vigorously.
- Incubate the tube on ice for 15-30 minutes.
- Centrifuge at >10,000 x g for 15 minutes at 4°C.



10. Carefully remove the supernatant without disturbing the pellet. The pellet contains the RNA. Discard the supernatant.
11. Wash the pellet with cold 80% ethanol. Centrifuge at  $>10,000 \times g$  for 15 minutes at 4°C. Carefully remove the supernatant without disturbing the pellet. The pellet contains the RNA. Discard the supernatant.
12. Air-dry the pellet. Resuspend the RNA in 7.5  $\mu\text{l}$  of Nuclease-Free Water or TE Buffer.

### B. Tobacco Acid Pyrophosphatase Treatment

Tobacco Acid Pyrophosphatase (TAP) removes the 5'-cap structure from 5'-capped RNA (e.g., eukaryotic mRNA) in the RNA sample. The end product of a TAP reaction is mRNA with a 5' monophosphate, which is required for the 5'-RACE Acceptor Oligo ligation reaction in Step C.

Required in Part B

Component Name	Tube Label	Cap Color
Tobacco Acid Pyrophosphatase	TAP Enzyme	Red
TAP Buffer	TAP Buffer	
RiboGuard RNase Inhibitor	RiboGuard RNase Inhibitor	

1. Assemble the following reagents on ice, in the order given, in a thin-walled PCR tube. Pipette each reagent several times to mix thoroughly before adding it to the tube.
 

1 $\mu\text{l}$	TAP Buffer
0.5 $\mu\text{l}$	RiboGuard RNase Inhibitor
7.5 $\mu\text{l}$	RNA sample (1-10 $\mu\text{g}$ )
1 $\mu\text{l}$	TAP Enzyme
10 $\mu\text{l}$	Total reaction volume
2. Incubate the reaction at 37°C for 30 minutes.
3. Remove the tube from the 37°C incubation and keep it at room temperature. Proceed immediately to Part C.

**C. 5'-RACE Acceptor Oligo Ligation**

This step ligates the 5'-RACE Acceptor Oligo (an oligoribonucleotide), which contains a PCR priming site, to the 5' end of the RNAs. Only those RNAs that have a 5' monophosphate will serve as substrate ("donor") for the ligation reaction.

Required in Step C

Component Name	Tube Label	Cap Color
T4 RNA Ligase	T4 RNA Ligase	Yellow
RNA Ligase Buffer	RNA Ligase Buffer	
5'-RACE Acceptor Oligo	5'-RACE Acceptor Oligo	
TAP STOP Buffer	TAP STOP Buffer	
2 mM ATP Solution	2 mM ATP	

**Important!** Be sure to add the reagents in the order they are listed here. Do NOT prepare a MasterMix tube when processing multiple samples because it is important that the TAP STOP Buffer be added before the 2 mM ATP Solution.

- Assemble the following reagents in the order given. Pipette each reagent several times to mix thoroughly before adding to the tube.

10 µl	TAP-treated RNA from Part B, Step 3
4 µl	Nuclease-Free Water
2 µl	RNA Ligase Buffer
1 µl	TAP STOP Buffer
1 µl	5'-RACE Acceptor Oligo
1 µl	2 mM ATP Solution
1 µl	T4 RNA Ligase
20 µl	Total reaction volume

- Incubate the reaction at 37°C for 30 minutes.

## D. First-Strand cDNA Synthesis

In this step, 3'-polyadenylated RNAs are reverse-transcribed into cDNA. cDNA synthesis is primed by the cDNA Synthesis Primer, which consists of an oligo(dT) sequence with a PCR priming sequence (different from the PCR priming sequence of the 5'-RACE Acceptor Oligo) at its 5' end.

Required in Step D

Component Name	Tube Label	Cap Color
MMLV Reverse Transcriptase	MMLV Reverse Transcriptase	Blue
MMLV Reverse Transcriptase Buffer	MMLV RT Buffer	
cDNA Synthesis Primer	cDNA Synthesis Primer	
dNTP PreMix	dNTP PreMix	
RNase Solution	RNase Solution	
Nuclease-Free Water	Nuclease-Free Water	Clear

1. Add the following reagents to the reaction tube at room temperature. Pipette each reagent several times to mix thoroughly before adding to the tube.

20 µl	5'-RACE Acceptor Oligo-ligated RNA from Part C, Step 2
14 µl	Nuclease-Free Water
1 µl	cDNA Synthesis Primer
2 µl	dNTP PreMix
2 µl	MMLV RT Buffer
1 µl	MMLV Reverse Transcriptase
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40 µl	Total reaction volume

2. Incubate the reaction at 37°C for 1 hour.
3. Incubate the reaction at 85°C for 10 minutes to heat-inactivate the MMLV-RT. Then incubate the tube at 55°C for the downstream reactions.
4. Add 1 µl of RNase Solution to the reaction and incubate at 55°C for 5 minutes. The reaction should be kept at 55°C until the PCR amplification in Part E.

## E. Second-Strand cDNA Synthesis and PCR Amplification

The ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit uses a PCR amplification step to create both the second strand of the cDNA and then to amplify the double-stranded DNA. PCR primers and the FailSafe PCR PreMix E, which contains dNTPs, buffer, and betaine (a PCR enhancer), are provided with the kit. The PCR enzyme must be provided by the user. We strongly recommend the use of a proofreading enzyme.

PCR Primer 1 anneals to and primes PCR amplification from the 3' end of the cDNA and the PCR Primer 2 anneals to and primes PCR amplification from the 5' end of the cDNA. Transcript-specific primers can be substituted for either PCR Primer 1 or PCR Primer 2 if desired, but specific PCR conditions will have to be optimized by the user.

Required in Part E

Component Name	Tube Label	Cap Color
PCR Primer 1	PCR Primer 1	Blue
PCR Primer 2	PCR Primer 2	
FailSafe PCR Premix E	FailSafe PCR Premix E	
Nuclease-Free Water	Nuclease-Free Water	Clear

Additional Required Reagents (provided by the user)

Thermostable DNA polymerase (preferably with proofreading activity)

**Note:** Be sure to use PCR Primer 1 and PCR Primer 2 in the Blue-cap tubes in this Step.

1. Add the following reagents to the reaction tube at 55°C. Pipette each reagent several times to mix thoroughly before adding it to the tube.

41 µl	Nuclease-treated first-strand cDNA from Part D, Step 4
18 µl	Nuclease-Free Water
5 µl	PCR Primer 1
5 µl	PCR Primer 2
30 µl	FailSafe PCR Premix E
1 µl	(2.5 U) thermostable DNA polymerase with proofreading activity
100 µl	Total reaction volume

2. Cycle the sample in a thermocycler under the following conditions:

95°C for 30 seconds (initial denaturation)

followed by 18–21 cycles of:

95°C for 20 seconds

60°C for 20 seconds

72°C for 3 minutes

Hold at 4°C.

## 9. Appendix

### Appendix A: Eukaryotic mRNA 5'- & 3'-RACE Kit Control Reactions

The ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit includes HeLa Total RNA Control (200 ng/µl) and β-actin PCR primers to test the performance of the kit. When performing the control reaction, use 1 µg (5 µl) of the HeLa Total RNA Control. Follow Part A, Step 1–Part E, Step 2. Then perform the 5'- and 3'-RACE reactions.

5'- & 3'-RACE Reactions. PCR primers are provided for 5' RACE and 3' RACE of human β-actin mRNA. Amplicons can be visualized on a 1% agarose gel. A thermostable DNA Polymerase, preferably with proofreading activity, must be provided by the user.

**5'-RACE Control Reaction**

The 5'-RACE control reaction uses the PCR Primer 1 and the  $\beta$ -Actin Reverse Primer (5' AGGTGTGGTGCCAGATTTTC 3') provided in the kit. The 5'-RACE control reaction produces an amplicon of ~400 bp.

1. Add the following reagents to the reaction tube. Pipette each reagent several times to mix thoroughly before adding it to the tube.

1 $\mu$ l	dsDNA from Part E, Step 2	
21 $\mu$ l	Nuclease-Free Water	(Clear-cap tube)
1 $\mu$ l	PCR Primer 1	(Blue-cap tube)
1 $\mu$ l	$\beta$ -Actin Reverse Primer	(Clear-cap tube)
25 $\mu$ l	FailSafe PCR Premix E	(Blue-cap tube)
49 $\mu$ l	Total volume	

2. Incubate the tube in a thermocycler. Once the temperature reaches 95°C, pause the cyclor and add 1  $\mu$ l (2.5 U) of thermostable DNA polymerase (provided by the user) to the tube.

Cycle the sample in a thermocycler using the following conditions:

95°C for 30 seconds (initial denaturation)

followed by 35 cycles of:

94°C for 20 seconds

60°C for 20 seconds

72°C for 20 seconds

Hold at 4°C.

**3'-RACE Control Reaction**

Dilute the amplified dsDNA from Part E, Step 2 1:100 with Nuclease-Free Water prior to the 3'-RACE control reaction. The 3'-RACE control reaction uses the PCR Primer 2 and the  $\beta$ -Actin Forward Primer (5' TTTGAATGATGAGCCTTCGTGCCC 3') provided in the kit. The 3'-RACE control reaction produces a smear from ~250-300 bp. The smear is the result of the different lengths of the poly(A) tail on the  $\beta$ -actin mRNAs in the HeLa RNA sample.

1. Add the following reagents to the reaction tube. Pipette each reagent several times to mix thoroughly before adding it to the tube.

1 $\mu$ l	dsDNA from Part E, Step 2 diluted 1:100	
21 $\mu$ l	Nuclease-Free Water	(Clear-cap tube)
1 $\mu$ l	PCR Primer 2	(Blue-cap tube)
1 $\mu$ l	$\beta$ -Actin Forward Primer	(Clear-cap tube)
25 $\mu$ l	FailSafe PCR Premix E	(Blue-cap tube)
49 $\mu$ l	Total volume	

2. Incubate the tube in a thermocycler. Once the temperature reaches 95°C, pause the cyclor and add 1 µl (2.5 U) of thermostable DNA polymerase (provided by the user) to the tube.

Cycle the sample in a thermocycler under the following conditions:

95°C for 30 seconds (initial denaturation)

followed by 35 cycles of:

94°C for 20 seconds

60°C for 20 seconds

72°C for 20 seconds

Hold at 4°C.

## Appendix B: PCR Primer 1 and PCR Primer 2 Sequences

The ExactSTART Eukaryotic mRNA 5'- & 3'-RACE Kit provides sufficient amounts of PCR Primer 1 and PCR Primer 2 to perform a total of 50 PCR amplifications. If additional primers are needed, the following oligodeoxyribonucleotides can be ordered from a supplier of choice:

### PCR Primer 1

5' TCATACACATACGATTTAGGTGACACTATAGAGCGGCCGCCTGCAGGAAA 3'

### PCR Primer 2

5' TAGACTTAGAAATTAATACGACTCACTATAGGCGGCCACCG 3'

The  $T_m$  of each primer is >60°C.

Additional sequence can be added to the 5' end of each primer if desired. However, PCR reaction conditions, as described in Part E, may have to be altered accordingly.

## 10. Kit Components Available Separately

### APex™ Heat-Labile Alkaline Phosphatase (includes 10X Reaction Buffer)

AP49010	10 Reactions
AP49050	50 Reactions
AP49100	100 Reactions

### Tobacco Acid Pyrophosphatase (includes 10X Reaction Buffer)

T19050	50 Units
T19100	100 Units
T19250	250 Units
T19500	500 Units

### T4 RNA Ligase (includes 10X Reaction Buffer and ATP Solution)

LR5010	1,000 Units
LR5025	2,500 Units
LR5050	5,000 Units

**RiboGuard™ RNase Inhibitor**

RG90925	2,500 Units
RG90910K	10,000 Units

**FailSafe™ PCR 2X PreMix E**

FSP995E	2.5 ml
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**FailSafe™ PCR Enzyme Mix**

FSE51100	100 Units
FSE5101K	1,000 Units

**Additional ExactSTART™ Kit****ExactSTART™ Full-Length cDNA Library Cloning Kit**

ES0907	10 Reactions
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Construct a cloned library of full-length cDNA from Eukaryotic mRNAs.

Visit [www.epicentre.com/exactstart](http://www.epicentre.com/exactstart) to learn more about the ExactSTART Platform

**11. Related Products****MasterPure™ RNA Purification Kit**

MCR85102	100 Purifications
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The MasterPure RNA Purification Kit provides all reagents needed to purify total RNA from >10,000 eukaryotic cells. The kit is compatible with a wide variety of cell and tissue types.

**MasterPure™ Yeast RNA Purification Kit**

MPY03010	10 Purifications
MPY03100	100 Purifications

The MasterPure Yeast RNA Purification Kit provides all reagents needed to purify RNA from yeast cells without the use of organic solvents, such as phenol, or columns.

*\*Use of betaine in DNA polymerase reactions, including, but not limited to use for PCR or DNA sequencing, is covered by U.S. Patent No. 6,270,962, European Patent No. 0742838, German Patent No. DE4411588C1, and other patents or patent applications in the U.S. and other countries that are either assigned or exclusively licensed to Epicentre. Purchase of a product from Epicentre that contains betaine and a thermostable DNA polymerase is accompanied by a limited non-exclusive license for the purchaser to use the purchased product solely for life science research, whether the purchaser performs research in a not-for-profit or a for-profit organization. However, if the product does not contain a thermostable DNA polymerase in addition to betaine, all for-profit organizations require a license from Epicentre in order to use betaine or a product that contains betaine in DNA polymerase reactions for research applications, and not-for-profit organizations require a license if the product, the research, or the result of the research is transferred to or obtained for or on behalf of a for-profit organization. Licenses are also available to use betaine in DNA polymerase reactions for human or animal diagnostics, screening, or other fields of use. Please contact Epicentre for information related to such licenses.*

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