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Message*BOOSTER*™ Whole Transcriptome cDNA Synthesis Kit for qPCR

Cat. No. MBWT80510 – 10 Reactions

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1. Kit Contents

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

Component Name	Tube Label	Reactions	Tube Color
Message*BOOSTER* Whole Transcriptome Primers	WT Primers	15 µl	Red
RiboGuard RNase Inhibitor	RNase Inhibitor	10 µl	
Message*BOOSTER* Reverse Transcription PreMix	RT PreMix	50 µl	
Message*BOOSTER* DNA Polymerase PreMix	DNA Pol PreMix	60 µl	
Message*BOOSTER* DNA Polymerase	DNA Polymerase	10 µl	
Message*BOOSTER* cDNA Finishing Solution	Finishing Solution	10 µl	
MMLV Reverse Transcriptase	MMLV-RT	10 µl	
Random Primers	Random Primers	30 µl	Blue
RNase H	RNase H	10 µl	
NTP PreMix	NTP PreMix	150 µl	Green
Message*BOOSTER* T7 RNA Polymerase	T7 RNA Polymerase	25 µl	
Message*BOOSTER* T7 Transcription Buffer	T7 Transcription Buffer	25 µl	
RNase-Free DNase I	DNase I	30 µl	
Forward Control PCR Primer	Forward Control PCR Primer	10 µl	Yellow
Reverse Control PCR Primer	Reverse Control PCR Primer	10 µl	
HeLa Total RNA Control (40 ng/µl)	HeLa Total RNA Control	10 µl	
Dithiothreitol (DTT)	DTT	50 µl	Clear
RNase-Free Water	RNase-Free Water	1 ml	
Poly(I)	Poly(I)	20 µl	

Storage: Upon receipt of this kit, remove the tube containing the HeLa Total RNA Control and store it at –70°C to –80°C. Store the remainder of the kit at –20°C.

Additional Required Reagents and Equipment:

EpiScript™ Reverse Transcriptase (MMLV RNaseH- RT; Epicentre; optional, see Kit Overview)

SuperScript® III Reverse Transcriptase (Invitrogen Corp.; optional, see Kit Overview)

Thermocycler or water bath

Microcentrifuge

RNase-Free Water

RNA Purification columns such as: RNA Clean & Concentrator™-5 (Zymo Research) - or -

RNeasy® MinElute® Cleanup Kit (Qiagen)

Performance Specifications and Quality Control

The Message*BOOSTER* Whole Transcriptome cDNA Synthesis Kit for qPCR is function-tested using the HeLa Total RNA Control and PCR primer pairs specific for the 5′-region and 3′-region of β-actin mRNA. cDNA produced by the kit from 40 pg of HeLa Total RNA Control demonstrates a 3′/5′ ratio of 0.5-1.5. Melt curve analysis demonstrates a single sharp peak with a T_m ~88°C.

2. Kit Overview

A. **Round-One, First-Strand cDNA Synthesis:** The RNA in a total RNA sample is reverse-transcribed into first-strand cDNA. The reverse transcription reaction is primed using the proprietary Message*BOOSTER* Whole Transcriptome Primers, a combination of oligo(dT) and a population of mixed-sequence primers each containing a bacteriophage T7 RNA polymerase promoter at the 5′ end. Reverse transcription is performed using the MMLV Reverse Transcriptase provided in the kit or, if desired, using EpiScript™ Reverse Transcriptase (MMLV RNaseH-; Epicentre) or SuperScript III Reverse Transcriptase (Invitrogen; provided by the user).

B. **Round-One, Second-Strand cDNA Synthesis:** Following second-strand synthesis, the resulting double-stranded DNA (dsDNA) contains a T7 transcription promoter in an orientation that that will generate antisense RNA (aRNA, also called cRNA) during the subsequent *in vitro* transcription reaction.

C. **Round-One, *in vitro* transcription:** High yields of aRNA are produced in a rapid *in vitro* transcription reaction that uses the dsDNA produced.

D. **Round-One, RNA Purification:** The aRNA produced is purified by spin-column (supplied by the user).

E. **Round-Two, cDNA Synthesis:** The purified aRNA is reverse-transcribed into first-strand cDNA using the MMLV Reverse Transcriptase provided in the kit or, if desired, using EpiScript Reverse Transcriptase (Epicentre) or SuperScript III Reverse Transcriptase (Invitrogen Corp.; provided by the user). The reaction is primed using random-sequence primers. Diluted aliquots of the cDNA produced can be used without further purification for end-point or real-time PCR.

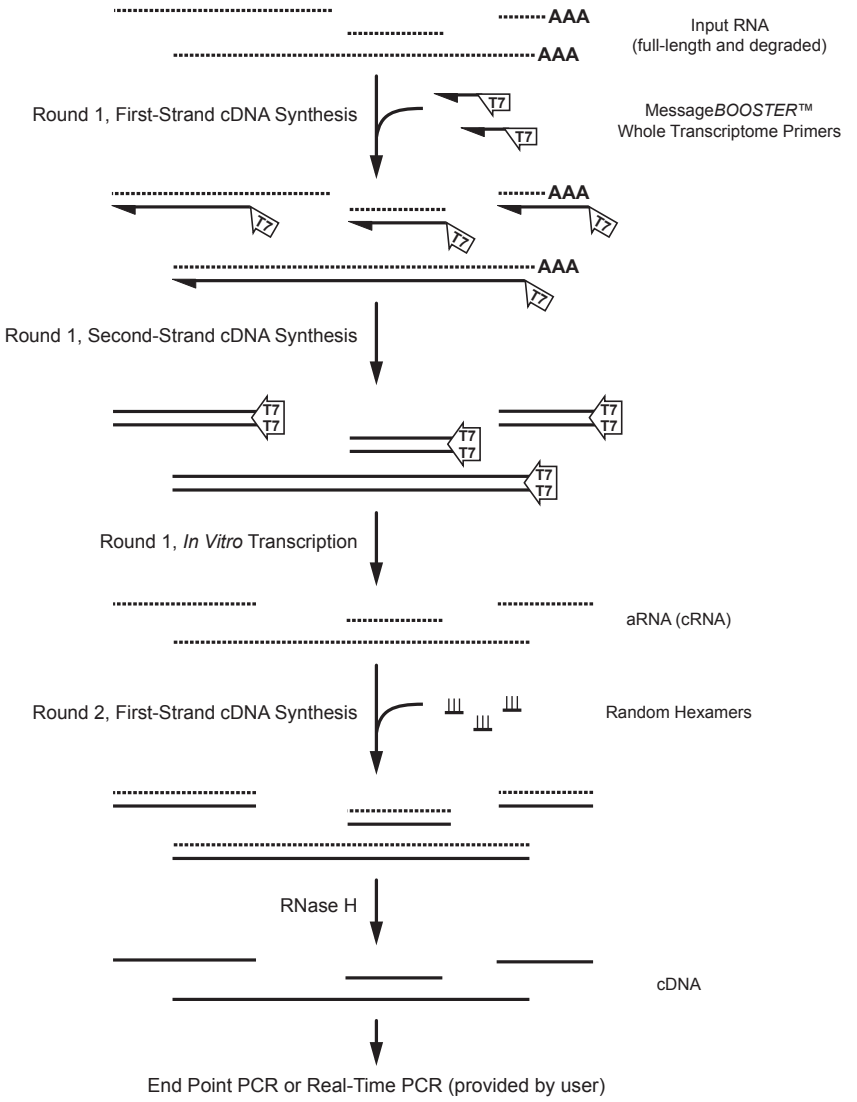

Figure 1. Message*BOOSTER*™ WT cDNA Synthesis Kit for qPCR Procedure.

3. Preparation

RNA Purification Methods and RNA Purity. Total cellular RNA, isolated by a number of methods, can be successfully amplified and converted to cDNA using the Message*BOOSTER* WT cDNA Synthesis Kit for qPCR. However, it is very important that the purified RNA be free of salts, metal ions, ethanol, and phenol that can inhibit the enzymatic reactions performed in RNA amplification and cDNA synthesis.

RNA Integrity. The Message*BOOSTER* WT cDNA Synthesis Kit for qPCR was developed for use with compromised or partially degraded RNA samples. For best results, we strongly recommend using an RNA sample composed of RNA fragments ≥150 bases in length. See Table 1.

RNA Isolated from FFPE Tissue. RNA isolated from FFPE tissue presents a particularly difficult challenge. The ability to amplify RNA from FFPE samples using the Message*BOOSTER* WT cDNA Synthesis Kit is dependent on factors that are often difficult for the user to control. We have observed that some RNA samples isolated from FFPE tissue have been successfully amplified and converted to cDNA with the kit, while other samples have failed. Therefore, your results may vary depending on the condition of the sample, age of the sample, and fixation method.

Maintaining an RNase-free Environment

Ribonuclease contamination is a significant concern for those performing RNA amplification. All components of the Message*BOOSTER* WT cDNA Synthesis Kit for qPCR have been tested to ensure the lack of contaminating ribonuclease activities and an RNase Inhibitor is provided in the kit. However, creating an RNase-free work environment and maintaining RNase-free solutions is critical for performing successful RNA amplification reactions. Therefore, we strongly recommend that you:

1) Use only RNase-free tubes and pipette tips in reactions containing RNA.
2) Always wear gloves when handling samples containing RNA. Change gloves frequently especially after touching potential sources of RNase contamination such as door knobs, pens, pencils, and human skin.
3) Always wear gloves when handling kit components. Do not pick up any kit component with an ungloved hand.
4) Keep all kit components tightly sealed when not in use. Keep all tubes containing RNA tightly sealed during the incubation steps.

Quality and Amount of Input Total RNA

The amount of input total RNA for a Message*BOOSTER* WT Kit reaction is dependent on the quality of the RNA. The quality of the input RNA can be determined using the 2100 BioAnalyzer (Agilent) with the RNA 6000 Nano LabChip® or the RNA 6000 Pico LabChip. Table 1 offers guidelines for the amount of input total RNA for three different qualities of RNA and judged by the 2100 BioAnalyzer. Electropherograms of "partially" degraded and "severely" degraded RNA samples are presented in Fig. 2. In all cases, we strongly recommend using an RNA sample that contains RNA fragments ≥150 bases in length.

Table 1. The amount of total RNA for a Message*BOOSTER* WT Kit reaction is dependent on the quality of the RNA.

RNA Quality	Approximate Size Distribution of the RNA Fragments	Minimum Amount of Input Total RNA
Intact	Full-length as judged by intact 28S and 18S rRNA	50 pg total RNA
Partially degraded	500 – 4000 bases	1 ng total RNA
Severely degraded	150 – 1000 bases	50 ng total RNA

Figure 2. Electropherograms produced by the Agilent 2100 BioAnalyzer of "partially" degraded (2A) and "severely" degraded (2B) RNA samples that were successfully amplified and converted to cDNA using the Message*BOOSTER* WT cDNA Synthesis Kit for qPCR.

Importance of Running the Message*BOOSTER* WT Kit Control Reaction

We strongly recommend that those who are not experienced with the Message*BOOSTER* WT Kit perform a control reaction (Appendix 1) prior to using a valuable sample. HeLa total RNA and Forward and Reverse qPCR Primers for detecting the 5′ region of the human β-actin mRNA are included in the kit.

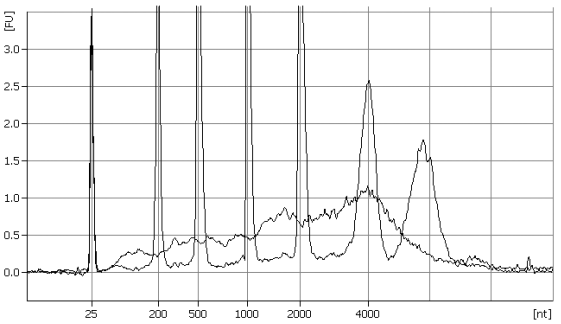

Fig. 2A. Trace of Partially Degraded RNA (overlapped with molecular weight ladder trace).

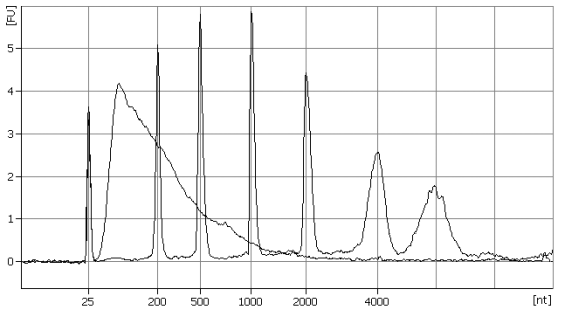

Fig. 2B. Trace of Severely Degraded RNA (overlapped with molecular weight ladder trace).

Control PCR Primers

Forward and reverse control PCR primers are supplied in the kit in yellow-cap tubes. The primers will amplify a 143-bp region from position 215 to 357 of the 1,793-b β-actin mRNA (see Fig. 3, below).

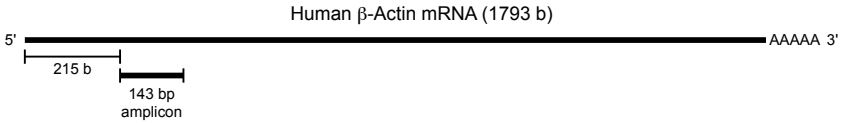

Figure 3. The β-actin control RT-PCR Map Position.

Performing the Message*BOOSTER* WT Kit Reaction

The Message*BOOSTER* Whole Transcriptome cDNA Synthesis Kit for qPCR includes many reagents. Before starting the procedure, please read it carefully; familiarize yourself with each kit component and in which step of the process it is used. Be sure to wear gloves when handling the kit components.

We recommend that all reactions be performed in sterile 0.2 ml thin-walled tubes using sterile pipette tips and recently calibrated pipettors. Very small volumes of some kit components are required for each reaction. Therefore, we recommend the user prepare Master Mixes of reaction components when amplifying multiple samples.

Some Simple but Important Factors for Obtaining Optimal Results

1) Familiarize yourself with the kit by running a control reaction (Appendix 1) before using a valuable sample.
2) Assemble the *in vitro* transcription reaction (Part C) at room temperature. Do not exceed the *in vitro* transcription reaction time indicated in the procedure.
3) Optional stopping points are noted following the Round-One, Second-Strand cDNA Synthesis step (Part B) and after purification of the aRNA (Part D).

4. Message*BOOSTER* WT Kit Procedure

Please read through the Message*BOOSTER* WT Kit Procedure carefully before beginning. We strongly recommend that users who are not experienced with the kit perform a control amplification reaction (Appendix 1) prior to using a valuable sample. HeLa Total RNA Control and PCR primers are provided with the kit.

A. Round-One, First-Strand cDNA Synthesis

The MMLV Reverse Transcriptase that is provided with the kit yields high quality results. However, if desired, EpiScript Reverse Transcriptase or Superscript III Reverse Transcriptase (Invitrogen; provided by the user) may be used. For best results, the RNA sample should be dissolved in RNase-Free Water. Please refer to "Quality and Amount of Input Total RNA" and "RNA Isolated from FFPE Tissue" for additional information on the amount of input total RNA to use.

Required in Part A

Component Name	Tube Label	Tube Color
Message*BOOSTER* Whole Transcriptome Primers	WT Primers	Red
Message*BOOSTER* Reverse Transcription PreMix	RT PreMix	
RiboGuard RNase Inhibitor	RNase Inhibitor	
MMLV Reverse Transcriptase	MMLV-RT	
Dithiothreitol	DTT	Clear
RNase-Free Water	RNase-Free Water	

Note: *EpiScript Reverse Transcriptase ((MMLV RNaseH-; Epicentre) or SuperScript III Reverse Transcriptase (provided by user) can be used in place of the MMLV Reverse Transcriptase included in the kit.*

Incubation temperatures performed in Part A: 50°C and 65°C.

1. Anneal the Message*BOOSTER* Whole Transcriptome Primers to the RNA sample. If a "no template" control reaction is performed, substitute RNase-Free Water for the total RNA sample.

   Important! *Be sure to use the MessageBOOSTER Whole Transcriptome Primers, in the red-cap tube, in this step.*

x	µl	RNase-Free Water
x	µl	Total RNA sample (Table 1, pg. 6 for guidelines)
0.5	µl	Message*BOOSTER* Whole Transcriptome Primers
3	µl	Total volume

2. Incubate at 65°C for 5 minutes in a water bath or thermocycler.
3. Chill on ice for 1 minute. Centrifuge briefly in a microcentrifuge.
4. Prepare the Round-One, First-Strand cDNA Synthesis Master Mix.

   For each first-strand cDNA synthesis reaction, combine on ice:

1.25	µl	Message*BOOSTER* Reverse Transcription PreMix
0.25	µl	DTT
0.25	µl	RiboGuard RNase Inhibitor
0.25	µl	MMLV-RT
2	µl	Total volume

   Note: *EpiScript Reverse Transcriptase or SuperScript III Reverse Transcriptase (provided by the user), can be used in place of the MMLV-RT that is supplied in the kit. If desired, use 50 U of either EpiScript or SuperScript III enzymes but DO NOT USE the DTT or the Reaction Buffer that is supplied with these enzymes.*

5. Gently mix the Round-One, First-Strand cDNA Synthesis Master Mix and then add 2 µl of it to each reaction.

6. If using MMLV Reverse Transcriptase (supplied in the kit) or EpiScript Reverse Transcriptase, incubate the reactions at room temperature for 10 minutes and then at 37°C for 60 minutes.

   If using SuperScript III Reverse Transcriptase, incubate the reactions at room temperature for 10 minutes and then at 50°C for 30 minutes.

B. Round-One, Second-Strand cDNA Synthesis

Required in Part B

Component Name	Tube Label	Tube Color
Message*BOOSTER* DNA Polymerase PreMix	DNA Pol PreMix	Red
Message*BOOSTER* DNA Polymerase	DNA Polymerase	Red
Message*BOOSTER* cDNA Finishing Solution	Finishing Solution	Red

Incubation temperatures performed in Step B: 37°C, 65°C and 80°C.

1. Prepare the Round-One, Second-Strand cDNA Synthesis Master Mix.

   For each second-strand cDNA synthesis reaction, combine on ice:

4.5 µl	Message*BOOSTER* DNA Polymerase PreMix
0.5 µl	Message*BOOSTER* DNA Polymerase
5.0 µl	Total volume

2. Gently mix the Round-One, Second-Strand cDNA Synthesis Master Mix and then add 5 µl of it to each reaction.
3. Gently mix the reactions and then incubate at 65°C for 10 minutes in a water bath or thermocycler. Centrifuge briefly in a microcentrifuge.

   Important! *Be sure to incubate the reactions at 65°C.*
4. Terminate the reactions by heating at 80°C for 3 minutes. Centrifuge briefly in a microcentrifuge then chill on ice.
5. Add 1 µl of Message*BOOSTER* cDNA Finishing Solution to each reaction.
6. Gently mix the reactions and then incubate at 37°C for 10 minutes.
7. Transfer the reactions to 80°C and incubate for 3 minutes. Centrifuge briefly in a microcentrifuge then chill on ice.

   Note: *If desired, the reactions can now be frozen and stored overnight at –20°C.*

C. Round-One, *In Vitro* Transcription

Required in Part C

Component Name	Tube Label	Tube Color
NTP PreMix	NTP PreMix	Green
Message*BOOSTER* T7 RNA Polymerase	T7 RNA Polymerase	
Message*BOOSTER* T7 Transcription Buffer	T7 Transcription Buffer	
RNase-Free DNase I	DNase I	
Dithiothreitol	DTT	Clear

Incubation temperatures performed in Step C: 37°C and 42°C.

1. Warm the Message*BOOSTER* T7 RNA Polymerase to room temperature. Thaw the remaining *in vitro* transcription reagents at room temperature. If a precipitate is visible in the thawed Message*BOOSTER* T7 Transcription Buffer, heat the Buffer to 37°C until it dissolves.
2. Thoroughly mix the thawed Message*BOOSTER* T7 Transcription Buffer.

   Important! *If a precipitate is visible in the thawed MessageBOOSTER T7 Transcription Buffer, heat the Buffer to 37°C until it dissolves. Mix the Buffer thoroughly.*
3. Prepare the *In Vitro* Transcription Master Mix.

   For each *in vitro* transcription reaction, combine at room temperature:

2	µl	Message*BOOSTER* T7 Transcription Buffer
14	µl	NTP PreMix
2	µl	DTT
2	µl	Message*BOOSTER* T7 RNA Polymerase
20	µl	Total volume

4. Gently mix the *In Vitro* Transcription Master Mix and then add 20 µl of it to each reaction.
5. Gently mix the reactions and then incubate at 42°C for 4 hours in a thermocycler or a water bath. If the thermocycler has a heated lid function, heat the lid only if the temperature of the lid can be maintained at about 42°C.

   Important! *Do not exceed 4 hour incubation. The 4 hour incubation gives optimal RNA yield and quality (length). Longer incubation times will result in lower quality RNA.*
6. Add 2 µl of RNase-Free DNase I to each reaction. Mix gently and then incubate at 37°C for 15 minutes.

D. Round One, aRNA Purification

We recommend using the RNA Clean & Concentrator-5 Kit (Zymo Research, provided by the user) for purifying the aRNA produced by the Message*BOOSTER* Whole Transcriptome cDNA Synthesis Kit for qPCR. This kit yields purified aRNA in a small volume that can be used directly in the Round-Two, cDNA Synthesis Step (Part E). Other RNA purification columns such as the RNeasy MinElute Cleanup Kit (Qiagen) can be used.

Required in Part D

Component Name	Tube Label	Tube Color
Poly(I)	Poly(I)	Clear

Note: *The user will need to provide RNase-Free Water for this purification step.*

1a. RNA purification using the RNA Clean & Concentrator-5 Kit (Zymo Research).

i. Add 1 µl of Poly(I) to each sample.

ii. Follow the manufacturer's directions for purifying total RNA including small RNAs.

iii. Elute the aRNA from the column using 8 µl of RNase-Free Water.

The eluted RNA can be used immediately in the Round-Two, First-Strand cDNA Synthesis step (Part E). If desired, the purified RNA can be quick frozen (e.g., dry ice/ethanol bath) and stored overnight at –70°C to –80°C.

1b. RNA purification using the Qiagen RNeasy MinElute Cleanup Kit (Qiagen). This procedure is slightly modified from the standard MinElute Cleanup Kit Procedure and requires that the user reduce the volume of the eluted RNA using a speed vacuum centrifugation prior to the Round-Two, First-Strand cDNA Synthesis step (Step E). If desired, the purified RNA can be quick frozen (e.g., dry ice/ethanol bath) and stored overnight at –70°C to –80°C.

i. Add 1 µl of Poly(I) to each sample.

ii. Follow the manufacturer's directions for sample application and washing.

iii. Elute the aRNA from the column using 14 µl of water. Then, reduce the volume of the aRNA to 3-8 µl using a Speed Vac Concentrator.

The purified concentrated RNA can be quick frozen (e.g., dry ice/ethanol bath) and stored overnight at –70°C to –80°C.

E. Round-Two, First-Strand cDNA Synthesis

The MMLV Reverse Transcriptase provided in the kit or EpiScript Reverse Transcriptase or SuperScript II Reverse Transcriptase (provided by the user) all produce comparable results when used in this step.

Required in Part E

Component Name	Tube Label	Tube Color
Message*BOOSTER* Reverse Transcription PreMix	RT PreMix	Red
MMLV Reverse Transcriptase	MMLV RT	
Random Primers	Random Primers	Blue
RNase H	RNase H	

Incubation temperatures performed in Step E: 37°C, 65°C and 95°C.

1. To each purified aRNA sample from Part D, add 2 µl of the Random Primers.
2. Incubate at 65°C for 5 minutes to anneal the Random Primers to the aRNA.
3. Chill on ice for 1 minute. Centrifuge briefly in a microcentrifuge.
4. Prepare the Round-Two, First-Strand cDNA Synthesis Master Mix.

   For each first-strand cDNA synthesis reaction, combine on ice:

1.5	µl	Message*BOOSTER* Reverse Transcription PreMix
0.25	µl	DTT
0.25	µl	MMLV-RT
2	µl	Total volume

   Note: *EpiScript or SuperScript III Reverse Transcriptase (provided by the user), can be used in place of the MMLV-RT that is supplied in the kit. If desired, use 50 U of either EpiScript or SuperScript III enzymes but DO NOT USE the DTT or the Reaction Buffer that is supplied with these enzymes.*
5. Gently mix the Round-Two, First-Strand cDNA Synthesis Master Mix and then add 2 µl of it to each reaction.
6. If using MMLV Reverse Transcriptase (supplied in the kit) or EpiScript Reverse Transcriptase, incubate the reactions at room temperature for 10 minutes and then at 37°C for 60 minutes.

   If using SuperScript III Reverse Transcriptase, incubate the reactions at room temperature for 10 minutes and then at 50°C for 30 minutes.
7. To each sample, add 0.5 µl of Message*BOOSTER* RNase H.
8. Gently mix each reaction and then incubate each at 37°C for 20 minutes.
9. Transfer the reactions to 95°C. Incubate each at 95°C for 2 minutes in a water bath or thermocycler.
10. Chill on ice for 1 minute. Centrifuge briefly in a microcentrifuge.

Note: *If desired, the cDNA samples can now be frozen and stored overnight at –20°C.*

Note: *If performing the MessageBOOSTER Control Reaction, continue with Appendix 2.*

F. Concentration and Yield of the cDNA

A Message*BOOSTER* reaction typically produces nanogram amounts of cDNA. Thus, it may be difficult to obtain an accurate measurement of the cDNA concentration and yield with even the most sensitive analytical instruments. However, before attempting to quantify cDNA concentration and yield, nucleotides and excess primers must be removed from the sample. The user should be sure to use a cleanup procedure that will accommodate nanogram amounts of single-stranded cDNA.

G. Assessing the Size of the cDNA Produced

If desired, the size of the single-strand cDNA produced can be analyzed using the Agilent 2100 Bioanalyzer: Dilute an aliquot of the cDNA with water to approximately 100 ng/µl. It is advisable to load and run duplicates of each sample tested. If a control reaction containing no input RNA was performed, load 1 µl of this sample as well. Analyze the samples following the manufacturer's instructions.

H. Guidelines for Real-Time qPCR Using cDNA Produced by a Message*BOOSTER* WT Reaction

PCR primer selection and amplicon size: Due to the nature of the RNA amplification and cDNA synthesis process used by the Message*BOOSTER* WT Kit, PCR primers can be chosen for any region of the transcript(s) of interest. Since the RNA put into a Message*BOOSTER* WT Kit reaction is expected to be fragmented (compromised or degraded), we recommend that the PCR primers be chosen so that the amplicon produced is 75-150 bp.

qPCR detection using fluorescent probes or SYBR® Green I Dye: Both fluorescent probes or SYBR Green I Dye have been used successfully. In general, fluorescent probes will provide better specificity for qPCR amplification.

Purification of the cDNA prior to qPCR: The cDNA produced by a Message*BOOSTER* reaction does not need to be purified prior to qPCR.

Amount of cDNA to add to the qPCR reaction: The amount of the cDNA to add to a qPCR amplification is dependent on the quality of the original RNA sample, amount of starting total RNA in the Message*BOOSTER* WT Kit reaction, and the abundance of the transcript of interest.

Use Table 2 as a guide to the amount of cDNA to use in qPCR. Dilutions of the cDNA should be made into water. In many cases, the optimal dilution of the cDNA will have to be determined experimentally. In general, the cDNA should be diluted less when detecting low-abundance transcripts, and more when detecting high-abundance transcripts.

Multiplex qPCR: Use Table 2 as a guide to the amount to the amount of cDNA to use for multiplex qPCR. However, multiplex qPCR is more challenging and may require more cDNA, as well as more rigorous optimization.

Table 2. Recommended amount of cDNA for qPCR.

Integrity of input total RNA in the Message*BOOSTER* WT reaction	Quantity of input total RNA in the Message*BOOSTER* WT reaction	Dilution of cDNA recommended
Intact RNA	50 pg (about 5 cell)	1:2 to 1:100
	250 pg	1:5 to 1:250
	500 pg	1:10 to 1:500
Partially degraded RNA	1 ng	1:2 to 1:100
	10 ng	1:10 to 1:250
	100 ng	1:20 to 1:500
Severely degraded RNA	50 ng	1:2 to 1:500
	100 ng	1:5 to 1:500
	500 ng	1:10 to 1:500

5. Appendix

Appendix 1: The Message*BOOSTER* WT Kit Control Reaction: RNA Amplification and cDNA Synthesis

The Message*BOOSTER* WT Kit provides 400 ng of HeLa total RNA at a concentration of 40 ng/µl.

Required for the Message*BOOSTER* WT Kit Control Reaction

Component Name	Tube Label	Tube Color
HeLa Total RNA Control (40 ng/µl)	HeLa Total RNA Control	Yellow
Message*BOOSTER* Whole Transcriptome Primers	WT Primers	Red
RNase-Free Water	RNase-Free Water	Clear

1. Thaw the HeLa Total RNA Control on ice.
2. On ice, dilute the thawed HeLa Total RNA Control 1:200 with RNase-Free Water by adding 1 µl of the HeLa Total RNA Control to 199 µl of RNase-Free Water. The concentration of the diluted HeLa Total RNA Control is now 200 pg/µl.
3. Anneal the Message*BOOSTER* WT Primers to the diluted HeLa Total RNA Control.

   The standard control reaction utilizes 200 pg of the HeLa Total RNA Control.

1 µl	RNase-Free Water
1 µl	Diluted HeLa Total RNA Control (200 pg)
1 µl	Message*BOOSTER* Whole Transcriptome Primers
3 µl	Total volume

4. Incubate the reaction at 65°C for 5 minutes in a water bath or thermocycler. While the reaction incubates, quick-freeze the remaining undiluted HeLa Total RNA Control, for example in a dry ice/ethanol bath and return it to –70°C to –80°C storage. Discard the diluted HeLa Total RNA Control.
5. Cool the annealing reaction on ice for at least 1 minute. Centrifuge the tube for 5-10 seconds to bring the sample to the bottom of the tube.
6. Continue the Control Reaction as described beginning in Part A, Step 4 through Part E. Then, perform the Message*BOOSTER* WT Kit qPCR Control Reaction as described in Appendix 2.

Appendix 2: The MessageBOOSTER WT Control Reaction: qPCR Control Reaction

Included in the Message*BOOSTER* WT Kit are the Forward and Reverse Control PCR primers for PCR amplification of a region near the 5′ end of human β-actin mRNA present in the HeLa Total RNA Control provided in the kit. A 143-bp amplicon will be produced.

Required for the Message*BOOSTER* WT qPCR Control Reaction

Component Name	Tube Label	Tube Color
Forward Control PCR Primer	Forward Control PCR Primer	Yellow
Reverse Control PCR Primer	Reverse Control PCR Primer	
RNase-Free Water	RNase-Free Water	Clear

1. Using the cDNA produced from the HeLa Total RNA Control in Part E, set up qPCR reactions using 1 µl of both the Forward Control PCR Primer and the Reverse Control PCR Primer in a 25 µl qPCR reaction.
2. Mix all the above content in PCR tubes and place them in a real-time PCR thermocycler. Use the cycling program recommended by the thermocycler and qPCR MasterMix manufacture. We recommend running a 40-cycle program using a two-step protocol, with annealing and extension at 60°C for 30 seconds. Melt curve analysis should be performed. Using the Control Primers included in the kit, melt curve analysis shows a sharp peak at T_m = 88°C.

6. Related Products

EpiScript Reverse Transcriptase (MMLV RNaseH-)

ERT12910K 10,000 Units

ERT12925K 25,000 Units

Additional Message*BOOSTER* Kits

Message*BOOSTER*™ cDNA Synthesis Kit for qPCR

MB060110 10 Reactions

MB060124 24 Reactions

This kit amplifies poly(A) RNA in a total RNA sample from as little as one cell and then converts it to amplified RNA then to cDNA for qPCR. The kit utilizes oligo(dT) to prime cDNA synthesis.

Message*BOOSTER*™ cDNA Synthesis from Cell Lysates Kit

MBCL9310 10 Reactions

This kit amplifies the poly(A) RNA directly from the lysate of 1-1000 cells without the need for RNA purification, and then converts the amplified RNA to cDNA for qPCR. The kit utilizes oligo(dT) to prime cDNA synthesis.

Kits for Amplifying and Labeling RNA for Microarrays

	TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101	TargetAmp™ 1-Round aRNA Amplification Kit 103	TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 105	TargetAmp™ Nano Labeling Kit for Illumina® Expression BeadChip®
Starting Total RNA	25-500 ng	25-500 ng	25-500 ng	25-500 ng
Time Required	1 Day	1 Day	1 Day	1 Day
End Product	Aminoallyl-aRNA	aRNA	Biotin-aRNA	Biotin-aRNA

	TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0	TargetAmp™ 2-Round aRNA Amplification Kit 2.0	TargetAmp™ 2-Round Biotin-aRNA Amplification Kit 3.0
Starting Total RNA	10-500 ng	10-500 ng	50-500 ng
Time Required	2 Days	2 Days	2 Days
End Product	Aminoallyl-aRNA	aRNA	Biotin-aRNA

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