epicentre®
an Illumina® company

MessageBOOSTER™ cDNA Synthesis Kit for qPCR

Cat. No. MB060110 – 10 Reactions

Cat. No. MB060124 – 24 Reactions

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1. Introduction

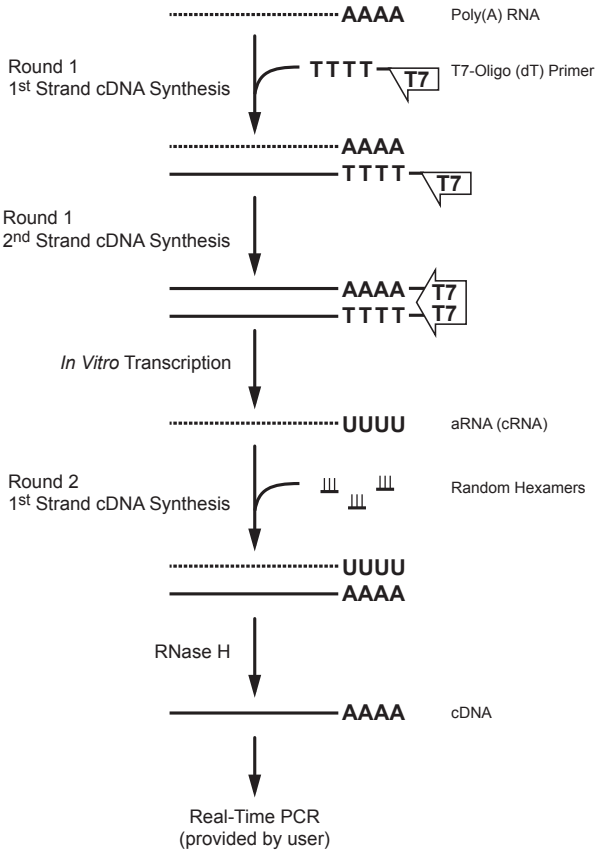

Figure 1. Message*BOOSTER*™ cDNA Synthesis Kit for qPCR Procedure.

The Message*BOOSTER*™ cDNA Synthesis Kit for qPCR reaction amplifies the poly(A) RNA (mRNA) in a total RNA sample isolated from 1-50 cells to enable more sensitive and more reproducible qPCR of even low-abundance transcripts.

A. **Round-One, 1st-strand cDNA Synthesis:** The poly(A) RNA component of a total RNA sample is reverse transcribed into first-strand cDNA. The reaction is primed from a T7-Oligo(dT) primer containing a phage T7 RNA Polymerase promoter sequence at its 5′ end. Superior results are obtained using SuperScript III Reverse Transcriptase (Invitrogen Corp.; provided by the user). The Message*BOOSTER* kit also contains MMLV Reverse Transcriptase which can be used in this step.

B. **Round-One, 2nd-strand cDNA Synthesis:** The RNA component of the cDNA:RNA hybrid is digested into small RNA fragments by RNase H. The RNA fragments then prime 2nd-strand cDNA synthesis. The resulting product is a double-stranded cDNA containing a T7 transcription promoter in an orientation that will generate anti-sense RNA (aRNA, also called cRNA) during the subsequent *in vitro* transcription reaction.

C. **Round-One, *In Vitro* Transcription:** High yields of aRNA (cRNA) are produced in a rapid *in vitro* transcription reaction that utilizes the double-stranded cDNA produced.

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

E. **Round-Two, 1st-strand cDNA Synthesis:** The purified aRNA (cRNA) is reverse transcribed into first-strand cDNA. In this step, comparable results are obtained using either Epicentre's MMLV Reverse Transcriptase (provided in the kit) or using SuperScript II Reverse Transcriptase or SuperScript III Reverse Transcriptase (Invitrogen Corp.; provided by the user). The reaction is primed using random sequence hexamer primers. Aliquots of the sense-strand cDNA produced can be used without further purification for real-time qPCR.

Performance Specifications and Quality Control

The Message*BOOSTER* cDNA Synthesis Kit for qPCR is function-tested using the NRK Total RNA Control and Control PCR Primers (specific for the low-abundance Porphobilinogen Deaminase [PBGD]) mRNA provided in the kit. One microliter of cDNA produced from 50 pg of NRK Total RNA Control yields a C_T value of <30 cycles (SYBR® Green I Dye detection) in a PCR reaction with the cycling conditions described in Appendix 2. Melt curve analysis demonstrates a single sharp peak with a T_m ~88°C.

2. Kit Contents

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

Component Name	Tube Label	Volume		Cap Color
		10 -rxn	24-rxn	
Message*BOOSTER* T7-Oligo(dT) Primer A	T7-Oligo(dT) Primer A	15 µl	30 µl	Red
RiboGuard RNase Inhibitor	RNase Inhibitor	10 µl	20 µl	
Message*BOOSTER* Reverse Transcription PreMix	RT PreMix	50 µl	90 µl	
Message*BOOSTER* DNA Polymerase PreMix 1	DNA Pol PreMix 1	60 µl	120 µl	
Message*BOOSTER* DNA Polymerase 1	DNA Polymerase 1	10 µl	18 µl	
Message*BOOSTER* cDNA Finishing Solution	Finishing Solution	15 µl	30 µl	
Message*BOOSTER* Random Primers	Random Primers	30 µl	60 µl	Blue
Message*BOOSTER* RNase H	RNase H	10 µl	20 µl	
MMLV Reverse Transcriptase	MMLV-RT	15 µl	20 µl	
Message*BOOSTER In Vitro* Transcription PreMix A	IVT PreMix A	300 µl	725 µl	Green
Message*BOOSTER* T7 RNA Polymerase	T7 RNA Polymerase	50 µl	120 µl	
Message*BOOSTER* T7 Transcription Buffer	T7 Transcription Buffer	50 µl	120 µl	
RNase-Free DNase I	DNase I	30 µl	60 µl	
Forward Control PCR Primer	Forward Control PCR Primer	10 µl	10 µl	Yellow
Reverse Control PCR Primer	Reverse Control PCR Primer	10 µl	10 µl	
Dithiothreitol (DTT)	DTT	60 µl	125 µl	Clear
RNase-Free Water	RNase-Free Water	1 ml	1 ml	
Poly(I)	Poly(I)	20 µl	40 µl	
NRK Total RNA Control (50 ng/µl)	NRK Total RNA Control	10 µl	10 µl	

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

Additionally Required Reagents and Equipment:

SuperScript® III Reverse Transcriptase (strongly recommended; Invitrogen Corp.)

SuperScript II Reverse Transcriptase (optional; Invitrogen Corp.)

Thermocycler or water bath

Microcentrifuge

RNase-Free Water

RNeasy® MinElute® Cleanup Kit

3. Preparation

The Quality of the Total RNA:

The success of an RNA amplification reaction is strongly influenced by the quality of the input total RNA. RNA quality has two components…purity of the RNA (or absence of contaminants) and integrity (intactness) of the RNA. If possible, the quality of the RNA should be assessed prior to every RNA amplification reaction. Poor quality RNA is the most common cause of sub-optimal Message*BOOSTER* Kit results!

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* cDNA Synthesis Kit for qPCR. However, it is very important that the purified RNA be free of salts, metal ions, ethanol, and phenol which can inhibit the enzymatic reactions performed in the RNA amplification and cDNA synthesis process.

RNA Integrity. Since cDNA synthesis is primed using an oligo(dT) primer, successful RNA amplification is dependent on an RNA sample that contains intact poly(A) RNA (e.g., RIN>7).

Maintaining an RNase-free Environment:

Ribonuclease contamination is a significant concern for those performing RNA amplification. All components of the Message*BOOSTER* 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 the user:

1) Autoclave all tubes and pipette tips that will be used 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.

Total RNA Input:

The Message*BOOSTER* cDNA Synthesis Kit for qPCR has been optimized for use with total RNA isolated from 1-50 cells (10-500 pg of total cellular RNA) per reaction. Amplifying >500 pg of total RNA in a single reaction may result in under-representation of some poly(A) RNA sequences in the cDNA produced. Therefore, if it is desirable to perform an amplification reaction using >500 pg of total RNA, we strongly recommend that the user perform multiple Message*BOOSTER* reactions, each containing up to but not exceeding 500 pg of total RNA.

Additional Suggestions:

Message*BOOSTER* cDNA Synthesis Kit for qPCR Control Reaction:

We strongly recommend that those who are not experienced with the Message*BOOSTER* cDNA Synthesis Kit for qPCR perform a control reaction (Appendix 1) prior to committing a precious sample. Normal Rat Kidney (NRK) total RNA and Forward and Reverse PCR primers for detecting a low abundance transcript Porphobilinogen Deaminase (PBGD) are provided.

Performing the Message*BOOSTER* cDNA Synthesis Kit for qPCR Reaction:

The Message*BOOSTER* cDNA Synthesis Kit for qPCR includes many reagents. Before starting, please read the procedure carefully and 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.

Choice of Reverse Transcriptase:

For optimal results, it is strongly recommended that SuperScript III Reverse Transcriptase (Invitrogen Corp.; provided by the user) be used for the Round One, 1st-Strand cDNA Synthesis reaction. The MMLV Reverse Transcriptase provided in the kit or SuperScript III Reverse Transcriptase or SuperScript II Reverse Transcriptase (provided by the user) all provide comparable results when used in the Round Two, 1st-Strand cDNA Synthesis reaction.

Some Simple but Important Factors for Obtaining Optimal Results:

1) Familiarize yourself with the kit by running a control reaction (Appendix 1) before committing a precious sample.
2) Use total RNA from no more than 50 cells (approximately 500 pg of total RNA) per reaction.
3) Assemble the two *in vitro* transcription reactions (Part C) at room temperature.

   Do not exceed the *in vitro* transcription reaction times indicated in the procedure.
4) Optional stopping points are noted following Round-One, 2nd-Strand cDNA Synthesis step (Part B) and after purification of the aRNA (Part D).

4. Message*BOOSTER* Kit Procedure

A. Round-One, 1st-strand cDNA Synthesis

For best results, SuperScript III Reverse Transcriptase (Invitrogen) is strongly recommended for use in this Step. The SuperScript III enzyme is provided by the user. MMLV Reverse Transcriptase is provided with the kit and may be used if desired. Both the SuperScript Reverse Transcriptases and MMLV-RT use the same Reverse Transcription PreMix. The total RNA sample must be free of contaminating salts, metal ions, ethanol, and phenol. For best results, the RNA sample should be dissolved in RNase-Free water.

Required in Part A

Component Name	Tube Label	Cap Color
Message*BOOSTER* T7-Oligo(dT) Primer A	T7-Oligo(dT) Primer A	Red
Message*BOOSTER* Reverse Transcription PreMix	RT PreMix	
RiboGuard RNase Inhibitor	RNase Inhibitor	
Dithiothreitol	DTT	Clear
RNase-Free Water	RNase-Free Water	
SuperScript III Reverse Transcriptase (provided by user) or MMLV Reverse Transcriptase	MMLV-RT	Blue

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

Important! *The MessageBOOSTER cDNA Synthesis Kit for qPCR has been optimized for use with total RNA isolated from 1-50 cells (about 10-500 pg of total cellular RNA) per reaction. Amplifying >500 pg of total RNA in a single reaction may result in under-representation of some poly(A) RNA sequences in the cDNA produced. Therefore, if it is desirable to perform an amplification reaction using >500 pg of total RNA, we strongly recommend that the user perform multiple reactions, each containing up to but not exceeding 500 pg of total RNA.*

1. Anneal the Message*BOOSTER* T7-Oligo(dT) Primer A 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 Message*BOOSTER *T7-Oligo(dT) Primer A in this Step.*

x	µl	RNase-Free Water
x	µl	Total RNA sample (10-500 pg)
1	µl	Message*BOOSTER* T7-Oligo(dT) Primer A
3	µl	Total

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, 1st-Strand cDNA Synthesis Master Mix.

   For each 1st-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	SuperScript III Reverse Transcriptase (200 U/µl; provided by the user)
– or –		
		MMLV-RT (provided in the kit)
2	µl	Total reaction volume

Important! *If using SuperScript III Reverse Transcriptase, do not use the SuperScript 5X Buffer or the DTT that is provided with the SuperScript III enzyme.*

5. Gently mix the Round-One, 1st-Strand cDNA Synthesis Master Mix and then add 2 µl of it to each reaction.
6. Gently mix the reactions. If using SuperScript III Reverse Transcriptase, incubate the reactions at 50°C for 30 minutes. If the thermocycler has a heated lid function, heat the lid only if the temperature of the lid can be maintained at about 50°C.

   If using MMLV Reverse Transcriptase, incubate the reactions at 37°C for 30 minutes. If the thermocycler has a heated lid function, heat the lid only if the temperature of the lid can be maintained at about 37°C.

B. Round-One, 2nd-strand cDNA Synthesis

Required in Part B

Component Name	Tube Label	Cap Color
Message*BOOSTER* DNA Polymerase PreMix 1	DNA Pol PreMix 1	Red
Message*BOOSTER* DNA Polymerase 1	DNA Polymerase 1	
Message*BOOSTER* cDNA Finishing Solution	Finishing Solution	

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

1. Prepare the Round-One, 2nd-Strand cDNA Synthesis Master Mix.

   For each 2nd-strand cDNA synthesis reaction, combine on ice:

4.5 µl	Message*BOOSTER* DNA Polymerase PreMix 1
0.5 µl	Message*BOOSTER* DNA Polymerase 1
5.0 µl	Total

2. Gently mix the Round-One, 2nd-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. Incubate the reactions 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	Cap Color
Message*BOOSTER In Vitro* Transcription PreMix A	IVT PreMix A	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 Round-One, *In Vitro* Transcription Master Mix.

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

4	µl	Message*BOOSTER* T7 Transcription Buffer
27	µl	Message*BOOSTER In Vitro* Transcription PreMix A
4	µl	DTT
4	µl	Message*BOOSTER* T7 RNA Polymerase
39	µl	Total

4. Gently mix the Round-One, *In Vitro* Transcription Master Mix and then add 39 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 each at 37°C for 15 minutes.

D. Round-One, aRNA Purification

This step uses the Qiagen RNeasy MinElute Cleanup Kit (Qiagen cat. no. 74204).

Use the RNase-Free Water that is provided in the MinElute Cleanup Kit.

Required in Part D

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

Note: *Use the RNase-Free Water provided in the RNeasy MinElute Cleanup Kit in this Step.*

1. Prepare 350 µl of RLT/β-ME Solution for each sample. Combine the RLT/β-ME in the ratio of 1 ml of Buffer RLT (provided in the MinElute kit) with 10 µl of β-ME (β-mercaptoethanol) as described in the MinElute kit handbook.
2. Prepare 650 µl of RPE Solution for each sample by diluting 1 volume of Buffer RPE (provided in the MinElute kit) with 4 volumes of 96-100% ethanol as described in the MinElute kit handbook.
3. To each sample add:

47.5	µl	RNase-Free Water
0.5	µl	Poly(I)
350	µl	RLT/β-ME Solution
250	µl	100% Ethanol

4. Apply each sample to an RNeasy MinElute spin column in a 2-ml collection tube. Centrifuge at >8,000 x g for 15 seconds. Discard the flow-through.
5. Apply 650 µl RPE Solution onto the column.
   Centrifuge at >8,000 x g for 15 seconds. Discard the flow-through.
6. Apply 650 µl 80% ethanol onto the column.
   Centrifuge at >8,000 x g for 15 seconds. Discard the flow-through.
7. Transfer the RNeasy MinElute spin column into a new collection tube.
   Centrifuge at full speed for 5 minutes.
8. Transfer the spin column to a 1.5-ml collection tube. Elute the aRNA by applying 15 µl of RNase-Free Water directly onto the center of the silica-gel membrane. Wait for 5 minutes. Centrifuge at full speed for 1 minute.

   Note: *If desired, the aRNA can now be quick frozen (e.g., dry ice/ethanol bath) and stored overnight at –70°C to –80°C.*

E. Round-Two, 1st-strand cDNA Synthesis

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

Required in Part E

Component Name	Tube Label	Cap Color
Message*BOOSTER* Reverse Transcription PreMix	RT PreMix	Red
Message*BOOSTER* Random Primers	Random Primers	Blue
Message*BOOSTER* RNase H	RNase H	
MMLV Reverse Transcriptase – or – SuperScript III or SuperScript II Reverse Transcriptase (provided by the user)	MMLV RT	

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

1. To each sample, add 2 µl of the Message*BOOSTER* Random Primers.
2. Transfer the entire volume of the purified aRNA from Step E1 into a 0.2-0.6 ml sterile reaction tube in which the remainder of the amplification reactions will be performed.
3. Adjust the volume of each aRNA sample to 3 µl by speed vacuum centrifugation without heat.

   Important: *Do not allow the RNA samples to completely dry.*

   Note: *Two suggestions are presented for efficiently performing the speed vacuum concentration step:*

   Before concentrating the RNA sample, add 3 µl of water to a separate reaction tube. Mark the level of the 3 µl of water with a marking pen. Then, concentrate the RNA sample using the speed vacuum centrifuge until it is at the same level in its (their) tube(s) as the 3-µl water sample.

   Use water samples to determine the time necessary to reduce the RNA samples to 3 µl. For example, if there are 4 RNA samples to concentrate, add a volume of water equal to the volume of the RNA samples to each of four separate tubes. Speed vacuum centrifuge the water sample(s) to 3 µl final volume. Record the time needed to reduce the water samples volume to 3 µl. Then, concentrate the RNA sample(s) using the speed vacuum centrifuge for the same amount of time as needed to reduce the volume of the water sample(s) to 3 µl.
4. Incubate at 65°C for 5 minutes in a water bath or thermocycler to anneal the Random Primers to the aRNA.
5. Chill on ice for 1 minute. Centrifuge briefly in a microcentrifuge.
6. Prepare the Round-Two, 1st-Strand cDNA Synthesis Master Mix.

   For each 1st-strand cDNA synthesis reaction, combine on ice:

   1.25 µl Message*BOOSTER* Reverse Transcription PreMix
   0.25 µl DTT
   0.25 µl RNase Inhibitor
   0.25 µl MMLV-RT (provided in the kit)
   – or –
   SuperScript III or SuperScript II Reverse Transcriptase (provided by the user)
   2 µl Total reaction volume

Important! *If using SuperScript III or SuperScript II Reverse Transcriptase, do not use the SuperScript 5X Buffer or the DTT that is provided with the SuperScript enzyme.*

7. Gently mix the Round-Two, 1st-Strand cDNA Synthesis Master Mix and then add 2 µl of it to each reaction.
8. Gently mix the reactions and then incubate each at room temperature for 10 minutes.
9. If using MMLV Reverse Transcriptase or SuperScript II Reverse Transcriptase, transfer the reactions to 37°C and incubate each at 37°C for 1 hour in a water bath or thermocycler. If the thermocycler has a heated lid function, heat the lid only if the temperature of the lid can be maintained at about 37°C.

   If using SuperScript III Reverse Transcriptase, transfer the reactions to 50°C and incubate each at 50°C for 1 hour in a water bath or thermocycler. If the thermocycler has a heated lid function, heat the lid only if the temperature of the lid can be maintained at about 50°C.
10. To each sample, add 0.5 µl of Message*BOOSTER* RNase H.
11. Gently mix each reaction and then incubate each at 37°C for 20 minutes in a water bath or thermocycler. If the thermocycler has a heated lid function, heat the lid only if the temperature of the lid can be maintained at about 37°C.
12. Transfer the reactions to 95°C.
    Incubate each at 95°C for 2 minutes in a water bath or thermocycler.
    Chill on ice for 1 minute.
    Centrifuge briefly in a microcentrifuge.

    Notes: *If desired, the reactions can now be frozen and stored overnight at –20°C. If performing the Message*BOOSTER *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 cDNA produced can be analyzed using the Agilent 2100 Bioanalyzer: Dilute an aliquot of the cDNA with water to approximately 100 ng/µl. Using the Agilent RNA 6000 Nano LabChip, load 1 µl of the diluted cDNA per well. 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. Run the samples per instrument procedure.

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

qPCR Primer Selection: Since the Message*BOOSTER* Kit uses oligo(dT)-primed cDNA synthesis, we recommend selecting PCR primers that prime within 500 bases of the 3′ end of the mRNA. Primers for sequences >500 bases from the 3′ end of the mRNA(s) may give reduced sensitivity.

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 cDNA produced by a Message*BOOSTER* reaction is in a volume of ~7 µl at the end of the standard Message*BOOSTER* reaction. The amount of the cDNA to add to a qPCR reaction is dependent on the amount of starting total RNA in the Message*BOOSTER* reaction and on the abundance of the transcript of interest.

Use the following Table as a guide to the amount of cDNA to use in the qPCR reaction.

Dilutions of the cDNA should be made into TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA).

Amount of starting total RNA in the MessageBOOSTER reaction	Detect low- or medium-abundance transcript	Detect high-abundance transcript
10 pg (about 1 cell)	1 µl of undiluted cDNA	1 µl of 1:10 diluted cDNA
100 pg (about 10 cells)	1 µl of 1:10 diluted cDNA	1 µl of 1:100 diluted cDNA
500 pg (about 50 cells)	1 µl of 1:50 diluted cDNA	1 µl of 1:1000 diluted cDNA

Low-abundance transcript = 1-1,000 copies per cell
Medium-abundance transcript = 1,000-10,000 copies per cell
High-abundance transcript = >10,000 copies per cell

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

5. Appendix

Appendix 1: The MessageBOOSTER cDNA Synthesis Kit for qPCR Control Reaction:

RNA Amplification and cDNA Synthesis

The Message*BOOSTER* kit provides 500 ng of Normal Rat Kidney (NRK) total RNA at a concentration of 50 ng/µl and forward and reverse PCR primers for the low abundance Porphobilinogen Deaminase (PBGD) mRNA.

Required for the Message*BOOSTER* Control Reaction

Component Name	Tube Label	Cap Color
NRK Total RNA Control (50 ng/µl)	NRK Total RNA	Clear
RNase-Free Water	RNase-Free Water	
Message*BOOSTER* T7-Oligo(dT) Primer A	T7-Oligo(dT) Primer A	Red

1. Thaw the NRK Total RNA Control on ice.
2. On ice, dilute the thawed NRK Total RNA Control 1:1000 with RNase-Free Water by adding 1 µl of the NRK Total RNA Control to 999 µl of RNase-Free Water. The concentration of the diluted NRK Total RNA Control is now 50 pg/µl.
3. Anneal the Message*BOOSTER* T7-Oligo(dT) Primer A to the diluted NRK Total RNA Control.

   The standard control reaction utilizes 50 pg of the NRK Total RNA Control.

1 µl	RNase-Free Water
1 µl	Diluted NRK Total RNA Control (50 pg)
1 µl	Message*BOOSTER* T7-Oligo(dT) Primer A
3 µl	Total reaction 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 NRK Total RNA Control, for example in a dry ice/ ethanol bath and return it to –70°C to –80°C storage. Discard the diluted NRK 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* Control qPCR Reaction as described in Appendix 2.

Appendix 2: The Message*BOOSTER* cDNA Synthesis Kit for qPCR Control Reaction:

The Message*BOOSTER* qPCR Control Reaction

Included in the Message*BOOSTER* cDNA Synthesis Kit for qPCR are the Forward and Reverse Control PCR primers for PCR amplification of the low-abundance Porphobilinogen Deaminase (PBGD) mRNA present in the NRK Total RNA Control provided in the Message*BOOSTER* Kit. Use of these Primers with cDNA produced in a Message*BOOSTER* from total RNA other than the provided NRK Total RNA Control may give unexpected results or results that are difficult to evaluate.

Required for the Message*BOOSTER* qPCR Control Reaction

Component Name	Tube Label	Cap 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 NRK 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 2-step protocol annealing and extending at 60°C for 30 seconds. Melt curve analysis should be performed.

6. Related Products

Kits for amplifying 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

Additional Message*BOOSTER* Kits

Message*BOOSTER*™ Whole Transcriptome cDNA Synthesis Kit for qPCR
MBWT80510 10 Reactions

Message*BOOSTER* Whole Transcriptome cDNA Synthesis Kit for qPCR provides all reagents needed to generate large amounts of cDNA from small amounts of compromised or degraded total
RNA Preparations for RT-PCR studies, qRT-PCR studies, or archival purposes.

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 Isolating Total Cellular RNA and mRNA

ArrayPure™ Nano-scale RNA Purification Kit
MPS04050 50 Purifications

The ArrayPure Kit provides all reagents needed to purify total RNA from 1-10,000 eukaryotic cells without the use of organic solvents such as phenol or columns.

7. Reference

1. Van Gelder, R. N. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* **87** (5), 1663.

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