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Message*BOOSTER*™ cDNA Synthesis from Cell Lysates Kit

Cat. No. MBCL90310 – 10 Reactions

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1. Introduction

Background

Quantitative reverse transcription-PCR (qRT-PCR) for gene expression studies from very small samples can be difficult for a number of reasons, including the following:

– Very few qRT-PCRs possible due to sample size.
– Lack of sensitivity, especially of low-abundance transcripts.
– Difficulty purifying minute amounts of total RNA.
– Need to collect samples frequently.
– Difficulty in archiving samples.

A Message*BOOSTER* cDNA Synthesis from Cell Lysates Kit reaction amplifies, in a linear manner, the mRNA (poly[A] RNA) directly from the lysates of 1-1,000 cells, without the need for isolating total cellular RNA. The amplified RNA is converted to cDNA that can be diluted and used for qPCR. Thus, a Message*BOOSTER* cDNA Synthesis from Cell Lysates Kit reaction eliminates the difficulty and uncertainty of purifying total RNA from minute samples while amplifying the mRNA for significantly improved qRT-PCR results.

2. Kit Contents

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

Component Name	**Tube Label**	**10 Reactions**	**Cap Color**
QuickExtract™ RNA Extraction Solution	QuickExtract™ RNA Solution	100 µl	Red
Message*BOOSTER*™ T7-Oligo(dT) Primer	T7-Oligo(dT) Primer	20 µl	
Message*BOOSTER*™ Reverse Transcription PreMix	RT PreMix	50 µl	
MMLV Reverse Transcriptase	MMLV-RT	10 µl	
RiboGuard™ RNase Inhibitor	RNase Inhibitor	10 µl	
Message*BOOSTER*™ DNA Polymerase PreMix	DNA Pol PreMix	90 µl	Blue
Message*BOOSTER*™ DNA Polymerase	DNA Polymerase	10 µl	
Message*BOOSTER*™ cDNA Finishing Solution	cDNA Finishing Solution	20 µl	
Message*BOOSTER*™ *In Vitro* Transcription PreMix	IVT PreMix	280 µl	Green
Message*BOOSTER*™ T7 RNA Polymerase	T7 RNA Polymerase	40 µl	
Message*BOOSTER*™ T7 Transcription Buffer	T7 Transcription Buffer	40 µl	
RNase-Free DNase I	RNase-Free DNase I	40 µl	
Message*BOOSTER*™ Random Primers	Random Primers	40 µl	Yellow
Message*BOOSTER*™ RNase H	RNase H	10 µl	
Dithiothreitol (DTT)	DTT	50 µl	Clear
RNase-Free Water	RNase-Free Water	1.2 ml	
Poly(I)	Poly(I)	15 µl	
Forward Control PCR Primer	Forward Control PCR Primer	10 µl	
Reverse Control PCR Primer	Reverse Control PCR Primer	10 µl	
Provided Separately			
NRK Total RNA Control (50 ng/µl)	NRK Total RNA Control	10 µl	Clear

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.

Additional Required Reagents and Equipment:

- Vortex mixer
- Thermocycler or water bath
- Microcentrifuge
- RNase-Free Water
- RNA Purification Columns such as:
  RNA Clean & Concentrator™-5 Kit (Zymo Research) or
  RNeasy® MinElute® Cleanup Kit (Qiagen)

Performance Specifications and Quality Control

The Message*BOOSTER* cDNA Synthesis from Cell Lysates Kit 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 in PCR with the cycling conditions described in APPENDIX 3. Melt-curve analysis demonstrates a single sharp peak with a T_m ~88°C.

3. Process Overview

The one-day reaction involves:

1. **Cell Lysis:** The harvested cells are lysed using the QuickExtract™ RNA Extraction Solution that is provided in the kit.
2. **Round-One, First-Strand cDNA Synthesis:** The Poly(A) RNA in the cell lysate is reverse-transcribed into first-strand cDNA. The reaction is primed by an oligo(dT) primer containing a phage T7 RNA Polymerase promoter sequence at its 5′ end.
3. **Round-One, Second-Strand cDNA Synthesis:** The single-strand cDNA produced in Step 2 is converted to double-strand cDNA that contains a T7 transcription promoter.
4. ***In Vitro* Transcription:** High yields of amplified RNA are produced in an *In Vitro* transcription reaction from the double-stranded cDNA template produced in Step 3.
5. **RNA Purification:** The amplified RNA is purified by spin-column chromatography (supplied by the user).
6. **Round-Two, First-Strand cDNA Synthesis:** The purified RNA is reverse-transcribed into first-strand cDNA. The reaction is primed using random-sequence hexamer primers. The cDNA produced can be diluted for qPCR using a qPCR system of the user’s choosing.

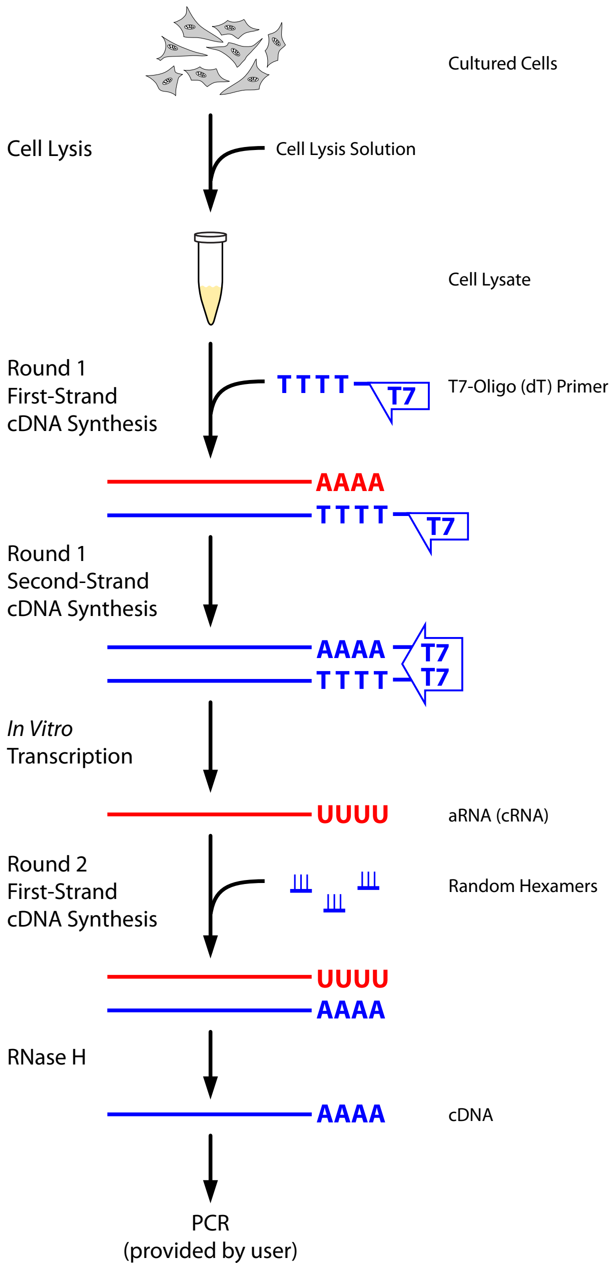

Figure 1. Overview of the Message*BOOSTER* cDNA Synthesis from Cell Lysates Kit Process.

4. Preparation

Maintaining an RNase-Free Environment:

Ribonuclease contamination is a significant concern for those performing RNA amplification and RT-PCR. 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) Use RNase-free (e.g., autoclaved) tubes and pipette tips.
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.

Familiarize Yourself with the Kit Components

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

Preparation of Cells

A kit reaction is optimized for use with 1-1,000 cells per reaction. Cultured cells, cells obtained by laser-capture microdissection, or flow-sorting methods, as well as biopsy and tissue samples can all be used successfully. Tissue samples must be dissociated for optimal results. Detach adherent cultured cells using standard laboratory procedure. If using proteolytic enzymes, for example trypsin, add additional serum, and collect the cells by centrifugation. Resuspend the cells in serum and count (or estimate) the desired number of cells and place them into a sterile 0.5-ml microcentrifuge tube. Pellet the cells and carefully aspirate off the serum. Proceed with Part A Cell Lysis or, if desired, wash the cells with ice-cold Phosphate-Buffered Saline (PBS). Centrifuge the washed cells and carefully aspirate the PBS. Place the cells on ice and continue with Part A Cell Lysis on page 9. We do not recommend using formalin-fixed paraffin-embedded (FFPE) samples.

The Cell Lysis Reaction

Cell lysis is done using the QuickExtract RNA Extraction Solution that is included in the kit. Three microliters of QuickExtract Solution will efficiently lyse 1-1,000 cells. If desired, you can perform the lysis using up to 10 µl of QuickExtract RNA Extraction Solution. However, a maximum of 3 µl is required per kit reaction. Therefore, if you perform the lysis is performed in a volume of >3 µl of QuickExtract RNA Extraction Solution, use just 3 µl of lysate for a reaction and store the remainder of the lysate at –70°C for future use.

Performing a Control Reaction

We strongly recommend that those users who are not experienced with the kit perform a control reaction (Appendices 2 & 3) prior to committing a valuable sample. Purified Normal Rat Kidney (NRK) total RNA and Forward and Reverse PCR primers for detecting a low-abundance transcript (PBGD) are provided.

Performing Multiple Reactions

Very small volumes of some kit components are required for each reaction. We recommend that you prepare Master Mixes of reaction components when performing multiple reactions simultaneously.

Choice of Reverse Transcriptase

MMLV Reverse Transcriptase is provided in the kit. However, other MMLV Reverse Transcriptase-type enzymes, provided by the user, such as SuperScript® II or SuperScript III (Invitrogen) can be used if desired. Modifications to the protocol for use of either of these reverse transcriptase enzymes are noted in the procedure.

The *In Vitro* Transcription Reaction

Assemble the *In Vitro* transcription reaction (Part D) at room temperature. Do not exceed the 4-hour *In Vitro* transcription reaction time indicated in the procedure.

"No Reverse Transcriptase" Control

The Message*BOOSTER* cDNA Synthesis from Cell Lysates Kit reaction includes a DNase treatment at Part D, Step 5 that will significantly reduce, but not completely eliminate, the cellular genomic DNA in the sample. Therefore, we recommend that a "no reverse transcriptase" (No-RT) control reaction be run for each sample. The kit includes sufficient reagents to run a "No-RT" control reaction for each reaction. It may not be necessary to perform the "No-RT" control if using PCR primers that span exon-exon junctions in the transcript(s) of interest.

qPCR and Primer Selection

The cDNA produced by the kit is ready to be diluted (see Appendix 1) for qPCR. qPCR is performed with the user's choice of qPCR system.

Since the 1st-strand cDNA reaction is done using an oligo(dT) primer, we recommend selecting PCR primers near the 3'-end of the mRNA.

Stopping Points

Optional stopping points are noted following the Round One, Second-Strand cDNA synthesis step (Part C) and after purification of the amplified RNA (Part E).

Message*BOOSTER* cDNA Synthesis from Cell Lysates Kit Procedure

Please read through the **Message*BOOSTER* cDNA Synthesis from Cell Lysates Kit** procedure carefully before beginning. We recommend that those users who are not experienced with the kit perform a control amplification reaction (Appendices 2 & 3; prior to committing a valuable sample. Normal Rat Kidney (NRK) Total RNA Control and PCR primers are provided with the kit.

A. Cell Lysis

Please see the notes Preparation of Cells and The Cell Lysis Reaction.

Required in Step A

Component Name	Tube Label	CapColor
QuickExtract™ RNA Extraction Solution	QuickExtract™ RNA Solution	Red

1. To dissociated pelleted cells, add 3-10 µl of QuickExtract RNA Extraction Solution.

   Important! *Just 3 µl of lysed cells are used for each reaction. Therefore, if the lysis is performed using >3 µl of QuickExtract RNA Extraction Solution, use just 3 µl of lysate in Part B, Step 1 and store the remainder of the lysate at –70°C to –80°C for future use.*

2. Lyse the cells by:

   a. Freezing at –70°C, on dry ice, or in liquid nitrogen and then thawing to room temperature. **- or -**

   b. Vigorous vortex mixing for 1 minute at room temperature.

3. Place the lysed cells on ice. If the lysis was done using exactly 3 µl of QuickExtract Solution, then proceed to Part B. If the lysis was done using more than 3 µl of QuickExtract Solution, then pipette 3 µl into a new sterile, 0.2-ml or 0.5-ml microfuge tube, and freeze the remaining lysate at –70°C to –80°C. Cell lysates can be stored at –70°C to –80°C for at least 2 months.

B. Round-One, First-Strand cDNA Synthesis

Choice of Reverse Transcriptase: MMLV Reverse Transcriptase is provided in the kit. However, other MMLV Reverse Transcriptase-based enzymes, provided by the user, such as SuperScript II or SuperScript III (Invitrogen) can be used. Modifications to the procedure for use of either of the SuperScript enzymes are noted in the procedure.

Required in Step B

Component Name	Tube Label	Cap Color
Message*BOOSTER*™ T7-Oligo(dT) Primer	T7-Oligo(dT) Primer	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	

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

1. Anneal the **Message*BOOSTER* T7-Oligo(dT) Primer** to the sample.

3	µl	Cell lysate
1	µl	Message*BOOSTER* T7-Oligo(dT) Primer
4	µl	Total reaction 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. Perform first-strand cDNA synthesis:

   Note 1: *If a "no Reverse Transcriptase" control reaction is performed, substitute RNase-Free Water for the Reverse Transcriptase enzyme.*

   Note 2: *If desired, substitute 0.25 µl of SuperScript II or SuperScript III Reverse Transcriptase (provided by the user) for the MMLV Reverse Transcriptase.* ***However, do not*** *use the SuperScript 5X Buffer or the DTT that is provided with the SuperScript enzymes.*

   To each reaction from Step B3, add:

1.25	µl	Message*BOOSTER* Reverse Transcription PreMix
0.25	µl	DTT
0.25	µl	RiboGuard RNase Inhibitor
0.25	µl	MMLV Reverse Transcriptase
6	µl	Total reaction volume

5. Gently mix the reactions. If using **MMLV Reverse Transcriptase or SuperScript II Reverse Transcriptase**, incubate the reactions at **37°C for 60 minutes**. If the thermocycler has a heated-lid function, heat the lid only if the temperature of the lid can be maintained at 37°C.

   If using SuperScript III Reverse Transcriptase, incubate the reactions at **50°C for 30-60 minutes**. If the thermocycler has a heated-lid function, heat the lid only if the temperature of the lid can be maintained at 50°C.

C. Round-One, Second-Strand cDNA Synthesis

Required in Step C

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

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

1. To each reaction from Step B5 (page 10), add:

4.5	µl	Message*BOOSTER* DNA Polymerase PreMix
0.5	µl	Message*BOOSTER* DNA Polymerase
11.0	µl	Total reaction volume

2. Gently mix the reaction(s) and then incubate at **65°C for 10 minutes** in a water bath or thermocycler. Centrifuge briefly in a microcentrifuge.
3. Terminate the reactions by heating at **80°C for 3 minutes**.

   Centrifuge briefly in a microcentrifuge, then chill on ice.
4. Add 1 µl of **Message*BOOSTER* cDNA Finishing Solution** to each reaction.
5. Gently mix the reactions and then incubate at **37°C for 10 minutes**.
6. Transfer the reaction(s) 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.*

D. *In Vitro* Transcription

Required in Step D

<table>
<tr><th>Component Name</th><th>Tube Label</th><th>Cap Color</th></tr>
<tr><td>MessageBOOSTER™ In Vitro Transcription PreMix</td><td>IVT PreMix</td><td rowspan="4">Green</td></tr>
<tr><td>MessageBOOSTER™ T7 RNA Polymerase</td><td>T7 RNA Polymerase</td></tr>
<tr><td>MessageBOOSTER™ T7 Transcription Buffer</td><td>T7 Transcription Buffer</td></tr>
<tr><td>RNase-Free DNase I</td><td>RNase-Free DNase I</td></tr>
<tr><td>Dithiothreitol</td><td>DTT</td><td>Clear</td></tr>
</table>

Incubation temperatures performed in Step D: 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.

   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.*
2. Thoroughly mix the thawed **Message*BOOSTER* T7 Transcription Buffer**.
3. To each reaction from Part C, Step 6, add:

2	µl	Message*BOOSTER* T7 Transcription Buffer
14	µl	Message*BOOSTER In Vitro* Transcription PreMix
2	µl	DTT
2	µl	Message*BOOSTER* T7 RNA Polymerase
32	µl	Total reaction volume

4. Gently mix the reaction(s) 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 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.*

5. Add **2 µl of RNase-Free DNase I** to each reaction. Mix gently and then incubate each reaction at **37°C for 15 minutes**.

E. RNA Purification

Purification of the amplified RNA is necessary prior to the final cDNA synthesis step. We recommend purification of the amplified RNA using the **RNA Clean & Concentrator™-5 columns** (Zymo Research, Cat. No. R1015; provided by the user). These columns elute the purified RNA in a volume that is compatible with the final cDNA synthesis step (Part F). Other commercial RNA purification columns can be used (e.g., RNeasy® MinElute® Cleanup Kit; Qiagen, Cat. No. 74204) but their use may require reducing the volume of the eluted RNA prior to the Round-Two, First-Strand cDNA Synthesis step.

If using the **RNA Clean & Concentrator-5 columns** (Zymo Research), follow the manufacturer's procedure for purifying *Total RNA including small RNAs* with the following suggestions:

- The volume of a Message*BOOSTER* reaction from Part D, Step 5 (above) is 34 µl. Therefore, in Step 1 of the RNA Clean & Concentrator-5 column procedure, **add 68 µl of RNA Binding Buffer** to each sample and mix.
- In Step 7 of the RNA Clean & Concentrator-5 column procedure, **elute the RNA using 8 µl of RNase-Free Water**. Apply the water directly onto the center of the column membrane. Wait 5 minutes. Centrifuge at full speed for 1 minute to collect the amplified RNA. The eluted RNA can be used immediately in Part F.

If using the **RNeasy MinElute Cleanup Kit** (Qiagen) columns:

- Add 0.5 µl of the Poly(I) (provided in the Message*BOOSTER* Kit) to each sample.
- Follow the manufacturer's directions for sample application and washing.
- Elute the RNA from the column using 14 µl of RNase-Free Water. Then, reduce the volume of the eluted RNA to 3-8 µl using a Speed-Vac® Concentrator. Do not let the sample dry.

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

F. Round-Two, First-Strand cDNA Synthesis

The purified RNA should be in a volume of 3-8 µl for optimal results. If the purified RNA is in a volume >8 µl, reduce the volume of the RNA using a Speed-Vac Concentrator. Do not let the sample dry.

MMLV Reverse Transcriptase is provided in the kit. However, other MMLV RT-type enzymes such as SuperScript II or SuperScript III (provided by the user) can be used. Modifications to the protocol for use of either of the SuperScript enzymes are noted in the procedure.

Required in Step F

Component Name	Tube Label	Cap Color
Message*BOOSTER*™ Reverse Transcription PreMix	RT PreMix	Red
MMLV Reverse Transcriptase	MMLV RT	
RiboGuard™ RNase Inhibitor	RNase Inhibitor	
Message*BOOSTER*™ Random Primers	Random Primers	Yellow
Message*BOOSTER*™ RNase H	RNase H	
Dithiothreitol	DTT	Clear

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

1. To each sample, add **2 µl** of the **Message*BOOSTER* Random Primers**.
2. Incubate at **65°C for 5 minutes** in a water bath or thermocycler to anneal the Random Primers to the RNA.
3. Chill on ice for 1 minute. Centrifuge briefly in a microcentrifuge.
4. To each reaction, add:

1.25	µl	Message*BOOSTER* Reverse Transcription PreMix
0.25	µl	DTT
0.25	µl	RiboGuard RNase Inhibitor
0.25	µl	MMLV Reverse Transcriptase

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

5. Gently mix the reaction(s) and then incubate each at **room temperature for 10 minutes**.
6. If using **MMLV Reverse Transcriptase** or **SuperScript II Reverse Transcriptase**, incubate the reaction(s) 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 37°C.

   If using **SuperScript III Reverse Transcriptase**, incubate the reaction(s) 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 50°C.

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** 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 37°C.
9. 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.

The cDNA produced, now in a volume of 7.5 µl-12.5 µl, does not need to be purified prior to qPCR. The cDNA can be diluted and used immediately for qPCR (see Appendix 1; below, for details) or stored at –20°C.

5. Appendices

Appendix 1

Guidelines for qPCR Using cDNA Produced by a MessageBOOSTER cDNA Synthesis from Cell Lysates Kit Reaction

Primer selection: Since the 1st-strand cDNA synthesis is done using an oligo(dT) primer, 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.

qPCR using fluorescent probes or fluorescent dye detection: Both fluorescent probes or fluorescent dyes can be used successfully.

Dilution of the cDNA for qPCR: The cDNA dilution factor is dependent on the number of cells used in the Message*BOOSTER* reaction and the abundance of the transcript of interest. Typically, 1 µl of diluted cDNA is used in a 25 µl qPCR.

Use the following table as a guide to diluting the cDNA for qPCR. Dilutions of the cDNA should be made into TE buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA; provided by the user).

Number of cells in the Message*BOOSTER* reaction	cDNA dilution factor
1-10 cells	1:2 to 1:100
10-50 cells	1:10 to 1:100
50 cells	1:50 to 1:1,000
>50 cells	1:100 to 1:1,000

Appendix 2

The Message*BOOSTER* cDNA Synthesis from Cell Lysates Kit Control Reaction:

RNA Amplification and cDNA Synthesis

The Message*BOOSTER* kit provides 500 ng of NRK total RNA at a concentration of 50 ng/µl and forward and reverse PCR primers for the low-abundance 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	
Forward Control PCR Primer	Forward Control PCR Primer	Yellow
Reverse Control PCR Primer	Reverse Control PCR Primer	
Message*BOOSTER*™ T7-Oligo(dT) Primer	T7-Oligo(dT) Primer	Red

1. Thaw the **NRK Total RNA Control** on ice.
2. On ice, dilute the thawed NRK Total RNA Control 1:1,000 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** to the **diluted NRK Total RNA Control**.

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

2	µl	RNase-Free Water
1	µl	Diluted NRK Total RNA Control (50 pg)
1	µl	Message*BOOSTER* T7-Oligo(dT) Primer
4	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 storage at –70°C to –80°C. 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 B, Step 4 through Part F. Then, perform the Message*BOOSTER* Control qPCR as described in Appendix 3 (next page).

Appendix 3

The Message*BOOSTER* cDNA Synthesis from Cell Lysates Kit Control Reaction:

The Message*BOOSTER* Control qPCR

Included in the Message*BOOSTER* cDNA Synthesis from Cell Lysates Kit are the Forward and Reverse Control PCR primers for PCR amplification of the low-abundance PBGD mRNA present in the NRK Total RNA Control. Use of these primers with cDNA produced from 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* Control qPCR

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

1. Using the cDNA produced from the NRK Total RNA Control in Part F, Step 9, to set up qPCRs. Mix the following reagents in PCR tubes:

9.5	µl	RNase-Free Water
1	µl	Forward Control PCR Primer
1	µl	Reverse Control PCR Primer
12.5	µl	2X qPCR MasterMix (provided by the user)
1	µl	cDNA
25	µl	Total reaction volume

2. Place the tubes in a real-time PCR thermocycler. Use the cycling program recommended by the thermocycler and qPCR MasterMix manufacturer. We recommend running a 40-cycle program using a two-step protocol annealing and extending at 60°C for 30 seconds. Melt-curve analysis should be performed.

   If using fluorescent dye detection, 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

Additional Message*BOOSTER* cDNA Synthesis Kits

Message*BOOSTER*™ cDNA Synthesis Kit for qPCR

MB060110 10 Reactions
MB060124 24 Reactions

This kit produces amplified cDNA from as little as 10 pg (about 1 cell) of *purified* total RNA for hundreds of sensitive qRT-PCR reactions.

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

MBWT80510 10 Reactions

This kit produces amplified cDNA from nanogram amounts of compromised or partially degraded total RNA.

Kits for Amplifying and Labeling Target RNA for Expression Microarray Studies

	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

Visit http://www.epicentre.com for details and ordering information.

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SuperScript is a registered trademark of Invitrogen Corp., Carlsbad, California.

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