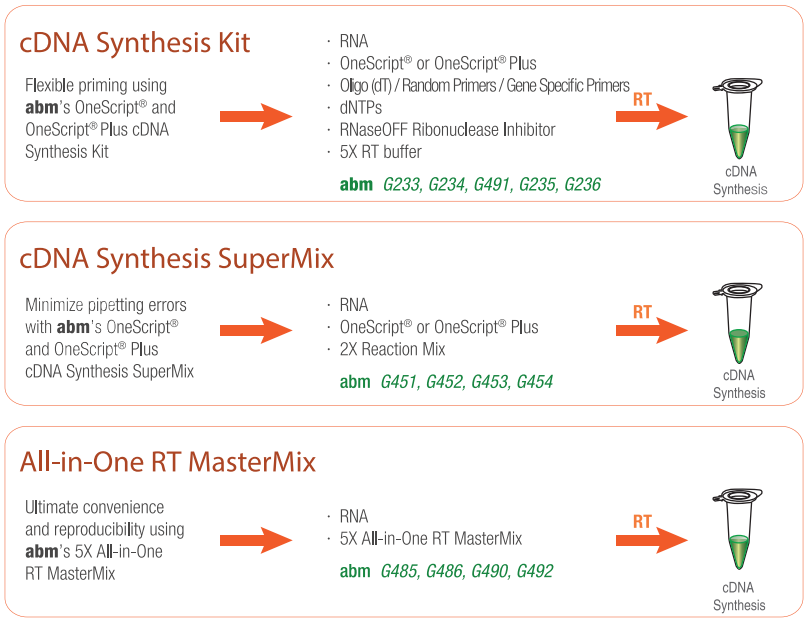
Real-Time PCR Set-up Instructions

(for **abm** Reverse Transcription Products)

The following protocol is for real-time PCR using **abm**'s EvaGreen qPCR MasterMix (Cat. No. MasterMix-R, -LR, -iC and -S). Please refer to the **EvaGreen qPCR MasterMix Selection Guide Version 1.0** (http://www.abmgood.com/Documents/files/EvaGreen qPCR MasterMix Selection Guide-Web.pdf) for selecting the appropriate qPCR MasterMix formulation applicable to your instrument model. The reverse transcription reaction prior to qPCR can be prepared from using any of the **abm**'s OneScript® and OneScript® Plus Reverse Transcriptase Products listed below.

Cat. No.	Product Name
G231, G232	OneScript® Reverse Transcriptase
G177, G237	OneScript® Plus Reverse Transcriptase
G233, G234, G491	OneScript® cDNA Synthesis Kit
G235, G236	OneScript® Plus cDNA Synthesis Kit
G451, G452	OneScript® cDNA Synthesis SuperMix
G453, G454	OneScript® Plus cDNA Synthesis SuperMix
G485, G486, G490, G492	5X All-in-One RT MasterMix

abm Reverse Transcription Selection Guide



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<Real-time Set-up using ABI StepOne System>

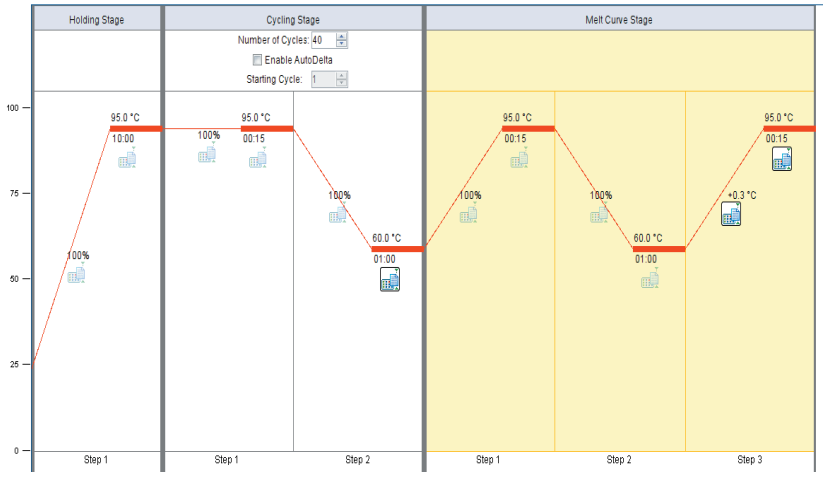
1. Thaw EvaGreen 2X qPCR MasterMix, template DNA, primers and nuclease-free water on ice. Mix each solution well.
2. Set up the following reaction mixture on ice:

Components	Volume	Final Concentration
EvaGreen 2X qPCR MasterMix*	10 µl	1X
Forward Primer (10 µM)	0.6 µl	300 nM
Reverse Primer (10 µM)	0.6 µl	300 nM
RT Product (cDNA)**	1 µl	< 100 ng
Nuclease-free H_2O	Up to 20 µl	-

* Recommended EvaGreen 2X qPCR MasterMix for ABI StepOne is MasterMix-R.

** If the gene of interest is highly expressed (i.e. House-Keeping Gene), it is best to dilute the cDNA at least 100 times before use. The volume of RT Reaction Solution (cDNA) can be adjusted to accommodate genes with low-expression; however the volume should not exceed 10% of the total PCR volume.

3. Start the qPCR Reaction. The Quantitation-Comparative CT (ΔΔCT) standard protocol as shown below is recommended. Optimize the reaction condition for primers with Tm higher or lower than 60°C.



Thermal Cycling Program

Temp	Duration	Cycle
Enzyme Activation		
95°C	10 mins	Hold
Denaturation, Annealing and Extension		
95°C	15 secs	40
60°C	60 secs	
Melting Curve		
95°C	15 secs	1
60°C	60 secs	
95°C	15 secs	

4. Check the amplification and melting curves after the reaction is completed. When using ABI StepOne System, please refer to its instruction manual for the analytical methods.

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<Real-time Set-up using Roche LightCycler480 System>

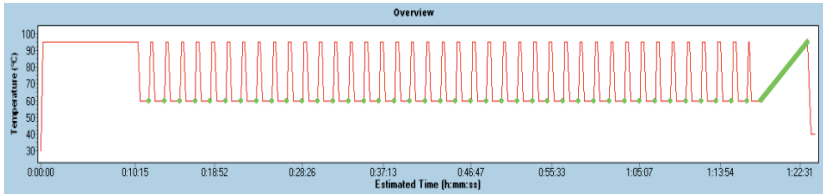
1. Thaw EvaGreen 2X qPCR MasterMix, template DNA, primers and nuclease-free water on ice. Mix each solution well.
2. Set up the following reaction mixture on ice:

Components	Volume	Final Concentration
EvaGreen 2X qPCR MasterMix*	10 µl	1X
Forward Primer (10 µM)	0.6 µl	300 nM
Reverse Primer (10 µM)	0.6 µl	300 nM
RT Product (cDNA)**	1 µl	< 100 ng
Nuclease-free H_2O	Up to 20 µl	-

* Recommended EvaGreen 2X qPCR MasterMix for Roche LightCycler480 is MasterMix-S.

** If the gene of interest is highly expressed (i.e. House-Keeping Gene), it is best to dilute the cDNA at least 100 times before use. The volume of RT Reaction Solution (cDNA) can be adjusted to accommodate genes with low-expression; however the volume should not exceed 10% of the total PCR volume.

3. Start the qPCR Reaction. The standard protocol as shown below is recommended. Optimize the reaction condition for primers with Tm higher or lower than 60°C.



Thermal Cycling Program

Temp	Duration	Cycle
Pre-Incubation		
95°C	10 mins	Hold
Amplification		
95°C	15 secs	40
60°C	60 secs	
Melting Curve		
95°C	15 secs	1
60°C	60 secs	
95°C	15 secs	

4. Check the amplification and melting curves after the reaction is completed. When using Roche LightCycler480 System, please refer to its instruction manual for the analytical methods.

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<Real-time Set-up using BioRad iQ5 Sytem>

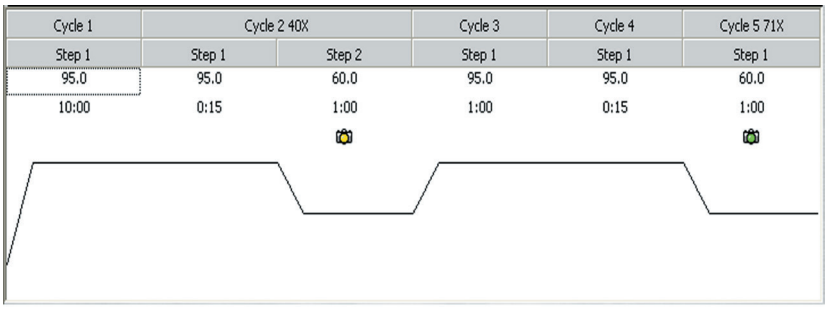
1. Thaw EvaGreen 2X qPCR MasterMix, template DNA, primers and nuclease-free water on ice. Mix each solution well.
2. Set up the following reaction mixture on ice:

Components	Volume	Final Concentration
EvaGreen 2X qPCR MasterMix*	10 µl	1X
Forward Primer (10 µM)	0.6 µl	300 nM
Reverse Primer (10 µM)	0.6 µl	300 nM
RT Product (cDNA)**	1 µl	< 100 ng
Nuclease-free H_2O	Up to 20 µl	-

* Recommended EvaGreen 2X qPCR MasterMix for BioRad iQ5 is MasterMix-iC.

** If the gene of interest is highly expressed (i.e. House-Keeping Gene), it is best to dilute the cDNA at least 100 times before use. The volume of RT Reaction Solution (cDNA) can be adjusted to accommodate genes with low-expression; however the volume should not exceed 10% of the total PCR volume.

3. Start the qPCR Reaction. The 40cycle+Melt standard protocol as shown below is recommended. Optimize the reaction condition for primers with Tm higher or lower than 60°C.



Thermal Cycling Program

Temp	Duration	Cycle
Pre-Incubation		
95°C	10 mins	Hold
Amplification		
95°C	15 secs	40
60°C	60 secs	

4. Check the amplification and melting curves after the reaction is completed. When using BioRad iQ5 System, please refer to its instruction manual for the analytical methods.

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<Real-time Set-Up using ABI 7300 Sytem>

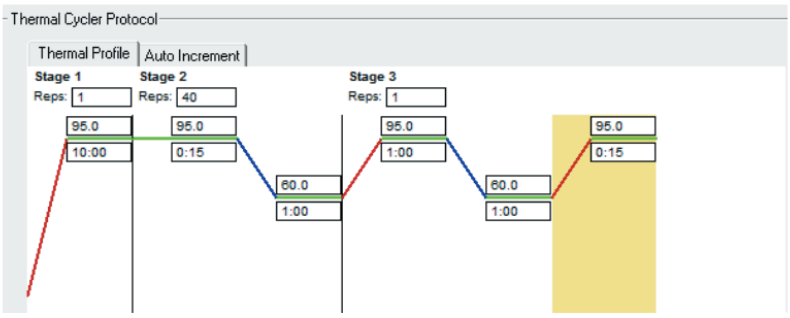
1. Thaw EvaGreen 2X qPCR MasterMix, template DNA, primers and nuclease-free water on ice. Mix each solution well.
2. Set up the following reaction mixture on ice:

Components	Volume	Final Concentration
EvaGreen 2X qPCR MasterMix*	10 µl	1X
Forward Primer (10 µM)	0.6 µl	300 nM
Reverse Primer (10 µM)	0.6 µl	300 nM
RT Product (cDNA)**	1 µl	< 100 ng
Nuclease-free H_2O	Up to 20 µl	-

* Recommended EvaGreen 2X qPCR MasterMix for ABI 7300 is MasterMix-R.

** If the gene of interest is highly expressed (i.e. House-Keeping Gene), it is best to dilute the cDNA at least 100 times before use. The volume of RT Reaction Solution (cDNA) can be adjusted to accommodate genes with low-expression; however the volume should not exceed 10% of the total PCR volume.

3. Start the qPCR Reaction. The standard protocol as shown below is recommended. Optimize the reaction condition for primers with Tm higher or lower than 60°C.



Thermal Cycling Program

Temp	Duration	Cycle
Pre-Incubation		
95°C	10 mins	Hold
Amplification		
95°C	15 secs	40
60°C	60 secs	
Melting Curve		
95°C	60 secs	1
60°C	60 secs	
95°C	15 secs	

4. Check the amplification and melting curves after the reaction is completed. When using ABI 7300 System, please refer to its instruction manual for the analytical methods.

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