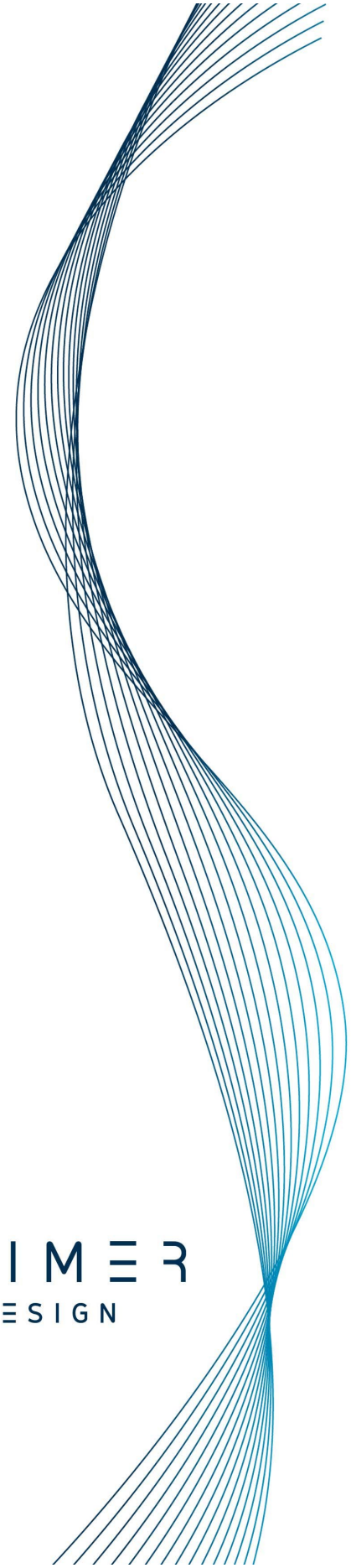
Internal RNA extraction control

Instructions for use of RNA real-time PCR internal extraction control kit



PRIMER DESIGN

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Introduction

When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the lysis buffer. This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process (internal extraction control). Successful co-purification and real-time PCR amplification of the control RNA also indicates that PCR inhibitors are not present at a high concentration.

A separate primer/probe mix are supplied with this kit to detect the exogenous RNA following cDNA synthesis using qPCR. The primers are present at PCR limiting concentrations which allows detection of the control cDNA and target cDNA in a multiplexing reaction. Amplification of the control cDNA does not interfere with detection of the target gene even when the target gene is present at low copy number. A number of different dyes are available so that a range of channels can be used to detect the control cDNA. A dye should be selected that reads through a separate channel to the target gene.

Channel	Excitation Wavelength	Emission Wavelength
FAM	495	520
Cy5	649	670
TAMRA	555	576
VIC	530	549

The exogenous RNA sequence supplied with this kit can be used with all human and animal tissues except rat and when used according to the protocols, gives a Cq value of 28±3.

Kit contents

- **Lyophilised Internal control RNA template (**150 reactions, **BLUE)**
- **Lyophilised Internal control primer/probe mix (**150 reactions, **BROWN)**
- **RNase/DNase free water (WHITE)**
- **Template preparation buffer (YELLOW)**
  for resuspension of internal control RNA template

Reagents and equipment to be supplied by user

- **Real-Time PCR instrument**
- **Master Mix or Master Mix components**
  This kit is designed to work well with all commercially available master mixes. However, we recommend the use of Primerdesign PrecisionPLUS OneStep 2X RT-qPCR Master Mix or oasig OneStep 2X RT-qPCR Master Mix.
  If intending to use this kit with a FAST Master Mix, please contact Primerdesign for further information, support@primerdesign.co.uk
- **Pipettors and Tips**
- **Vortex and centrifuge**

Kit storage

This kit is stable at room temperature but should be stored at -20ºC on arrival. Primerdesign does not recommend using the kit after the expiry date stated on the pack. Once the lyophilised components have been resuspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

Licensing agreement and limitations of use

PCR is covered by several patents owned by Hoffman-Roche Inc and Hoffman-LaRoche, Ltd. Purchase of Primerdesign kits does not include or provide license with respect to any patents owned by Hoffman-La Roche or others.

Primerdesign satisfaction guarantee

Primerdesign takes pride in the quality of all of our products. Should this product fail to perform satisfactorily when used according to the protocols in this manual, Primerdesign will replace the item free of charge.

Quality

At Primerdesign our commitment to Quality is a fundamental part of our business and we proactively make improvements in our service and product quality whilst meeting all relevant standards.

Notices and disclaimers

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GeneAmp®, TaqMan® and AmpliTaqGold® are registered trademarks of Roche Molecular Systems, Inc. The purchase of the Primerdesign™ reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Bench-side protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer/probe mix is in the base of the tube and is not spilt upon opening the tube.

2. **Resuspend lyophilised components in RNase/DNase free water provided.**

   To ensure complete resuspension, vortex each tube thoroughly, allow to stand for 5 minutes and vortex again before use.

Component – resuspend in water	Volume
Internal control primer/probe mix (**BROWN**)	165µl

3. **Resuspend internal control RNA template in the template preparation buffer provided.**

Component – resuspend in template preparation buffer	Volume
Internal control RNA template (**BLUE**)*	600µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

RNA extraction

The internal control can be added either to the RNA lysis/extraction buffer or into the RNA sample once it has been resuspended in lysis buffer.

DO NOT add the internal control RNA directly to the biological sample as this will lead to degradation and a loss in signal strength.

1. **Add 4µl of internal control RNA template (BLUE) to each sample in RNA lysis/extraction buffer**

2. **Complete RNA extraction according to the manufacturer's protocols**

One-step protocol

A one-step approach combines the reverse transcription and qPCR in a single closed tube. The protocols below are for the Primerdesign PrecisionPLUS OneStep 2X RT-qPCR Master Mix or oasig OneStep 2X RT-qPCR Master Mix. If you are using an alternative one-step product then follow the manufacturer's instructions.

For optimum performance and sensitivity all pipetting steps and experimental plate set up should be performed on ice. After the plate is poured proceed immediately to the one step amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. **For each RNA sample prepare a reaction mix according to the table below:**
   To perform an RT negative control, substitute PrecisionPLUS OneStep 2X RT-qPCR Master Mix for PrecisionPLUS Master Mix (which lacks the RT enzyme).

Component	1 reaction
PrecisionPLUS OneStep or oasig OneStep Master Mix	10µl
Target primer/probe mix	Xµl
Internal extraction control primer/probe mix (**BROWN**)	1µl
RNase/DNase Free water	Xµl
Final volume	**15µl**

2. **Pipette 15µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Prepare RNA templates for each of your samples in RNase/DNase free water.**
   Suggested concentration 5ng/µl.

4. **Pipette 5µl of RNA template into each well, according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

RT-qPCR amplification protocol

Amplification conditions using Primerdesign PrecisionPLUS OneStep 2X RT-qPCR Master Mix or oasig OneStep 2X RT-qPCR Master Mix

	Step	Time	Temp
	Reverse transcription	10 mins	55°C
	Enzyme activation	2 mins	95°C
X 40 cycles	Denaturation	10s	95°C
	DATA COLLECTION*	60s	60°C

* Fluorogenic data for the internal control RNA should be collected during this step through the channel specified in the introduction.

If intending to use this kit with a FAST cycling protocol, please contact Primerdesign for further information, support@primerdesign.co.uk

Interpretation of results

When used according to the above protocols, assuming a 100% extraction efficiency and adding an equivalent of 5µl of the extracted RNA sample to the PCR, then a Cq value of 28 is expected. However, this can vary significantly depending on the extraction efficiency, the quantity of RNA added to the RT and PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range.