

RealTime ready cDNA Pre-Amp Master and Pre-Amp Primer Pools

Introduction

cDNA pre-amplification in a multiplex PCR with target-specific primer pairs allows gene expression analysis of multiple targets when working with limited amounts of RNA (1 – 250 ng). The flexible RealTime ready gene expression workflow now features pre-amplification primer pools, containing matching primers for user-configured RealTime ready Custom Panels.

The primer pool is used with the **RealTime ready cDNA Pre-Amp Master** (see Figure 1). Pre-amplification of cDNA in a short multiplex PCR reaction with up to 96 different primer pairs will result in >4,000-fold amplification of the selected targets without creating a relevant bias to the original representation of each target in the input RNA.

The custom RealTime ready Pre-Amp Primer Pool is defined by configuring a RealTime ready Custom Panel on the Configurator (www.configurator.realtimeready.roche.com). Each primer pool is delivered in a volume of 500 µl (suitable for 40 pre-amplification reactions), containing equal amounts of primer pairs of all different assays selected for the panel.

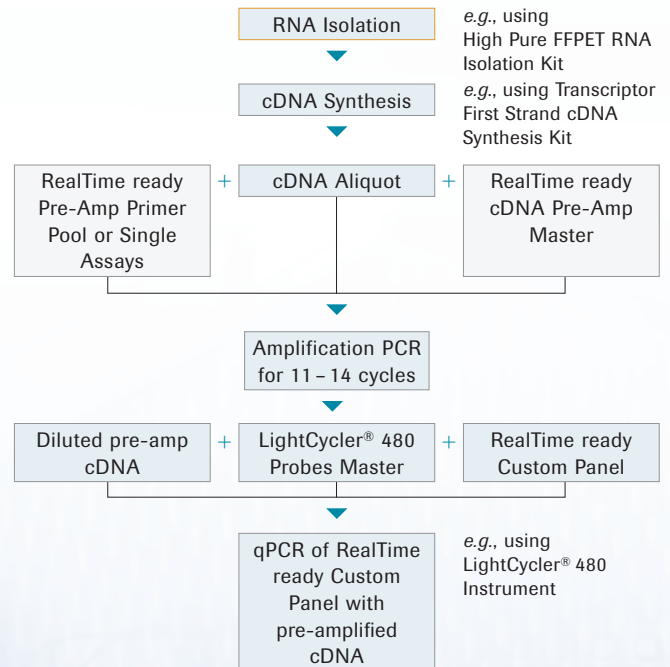


Figure 1: qPCR workflow including cDNA pre-amplification.

Typical Result

Obtain convenient and unbiased preamplification of qPCR targets (see Figure 2).

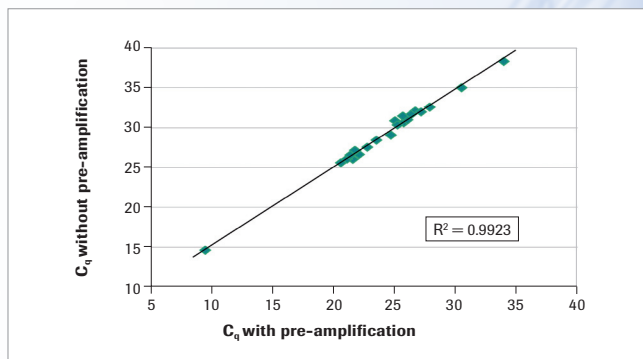


Figure 2: Unbiased results from pre-amplified cDNA. Total RNA was isolated from FFPE using the High Pure FFPE RNA Isolation Kit and reverse-transcribed using the Transcriptor First Strand cDNA Synthesis Kit. 5 ng of cDNA (pre-amplified vs. not pre-amplified) were subjected to qPCR on the LightCycler® 480 System.

Pre-amplified samples were 1:40 diluted before qPCR. Each data point reflects one target. Despite dilution, values of pre-amplified targets shift approximately 5 Cqs earlier. This substantial shift is highly reproducible for all targets analyzed.


RealTime ready Pre-Amp Primer Pool Ordering Workflow

Online ordering is available for Pre-Amp Primer Pools matching the assays on a Custom Panel for all layouts containing 96 or fewer different targets. To order primer pools from layouts that offer the possibility of more than 96 different targets (for example, 384-128, 384-192 and 384-384), please contact the local Roche sales representative.

- 1 Go to the RealTime ready Configurator (www.configurator.realtimeready.roche.com) and configure a custom panel with your selected layout.
- 2 Add the Custom Panel configuration to the shopping cart of the RTR Configurator. This will create a "Configuration Number" to use when ordering the custom panel and/or the matching primer pool.
- 3 The RealTime ready Pre-Amp Primer Pool (Cat. No. 06 725 309 001) is in the RealTime ready Shopping Cart under the "Pre-Amp Primer Pools" section. Paste the Configuration Number of the panel into the empty field, then click "Add to Shopping Cart".


Pre-Amp Primer Pools

One tube of 400 µl RealTime ready pre-amp primer mix solution for a custom panel defined by the configuration number. Enough for 40 x 50 µl cDNA pre-amplifications with the RealTime ready cDNA Pre-Amp Master.

Products	Configuration No.	Catalog No.	Price	
RealTime ready Pre-Amp Primer Pool	<input type="text"/>	06725309001	200,00 €	 Add to Shopping Cart

- 4 The selected Pre-Amp Primer Pool will then appear in the section "Pre-Amp Primer Pools Chosen", ready to order.

Pre-Amp Primer Pools Chosen

Products	Configuration No.	Catalog No.	Quantity	Price	Subtotal	
RealTime ready Pre-Amp Primer Pool	100039667	06725309001	<input type="text" value="1"/>	200,00 €	200,00 €	 Delete

Note:

Pre-Amp Primer Pools for a configuration containing Designer Assays must be ordered along with the corresponding Custom Panel.

For life science research only. Not for use in diagnostic procedures.

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