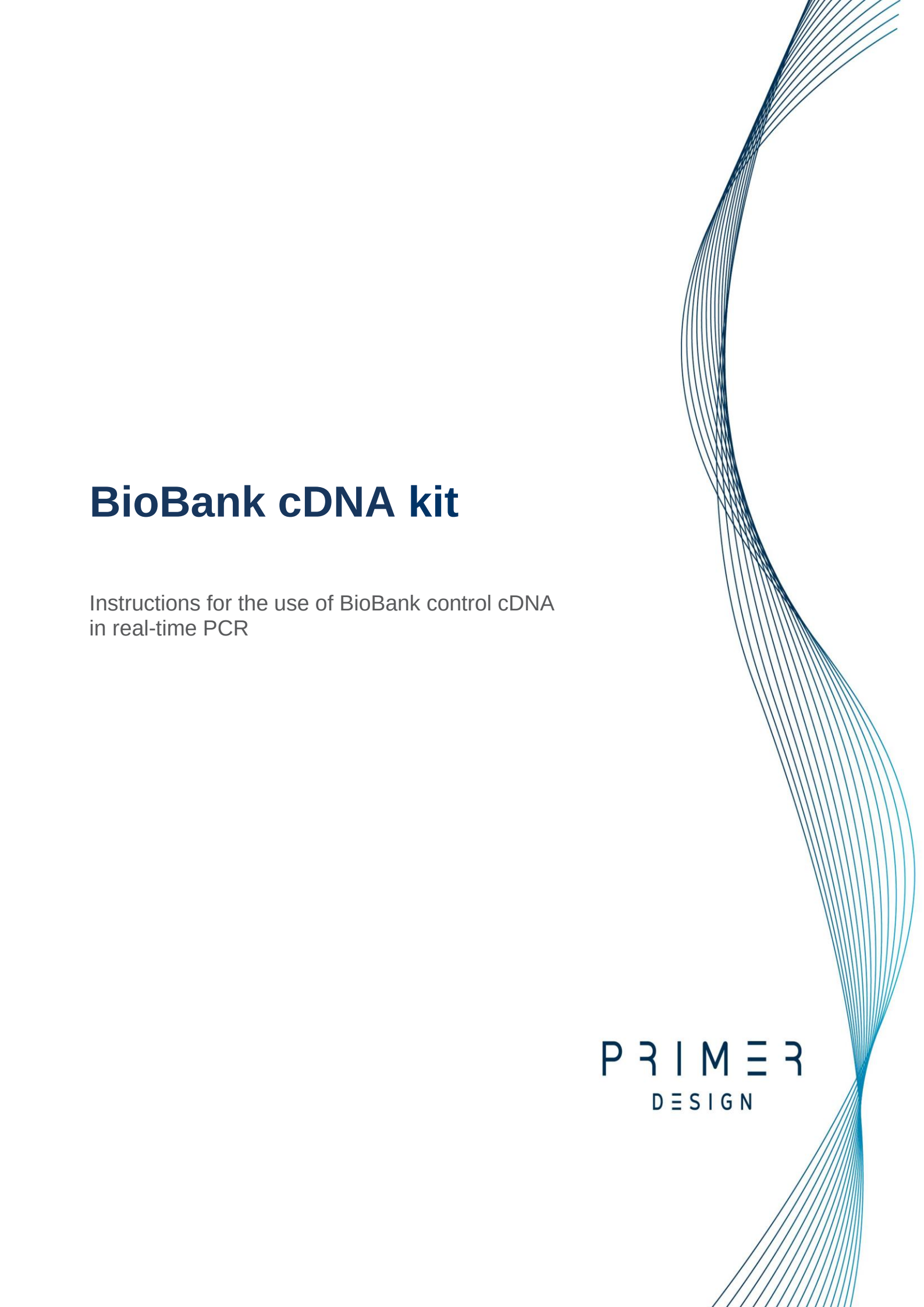
BioBank cDNA kit

Instructions for the use of BioBank control cDNA
in real-time PCR



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Introduction

The BioBank kit contains a high-quality source of cDNA template validated for use in real-time PCR experiments. The cDNA is reverse transcribed from high quality DNase treated RNA, from a variety of sources, using an optimised blend of Oligo(dT) and random nonamer primers. BioBank cDNA is therefore essentially free of genomic DNA and PCR inhibitors and covers the widest possible range of RNA and mRNA transcripts in the specified tissue or cell line. BioBank cDNA is useful for expression profiling of newly identified genes and as a positive control for the qPCR step.

Positive control primer/probe mix

A positive control primer/probe mix is supplied in the kit which detects 18S ribosomal RNA. These primer/probe mix includes a Hydrolysis probe which can be detected through the FAM channel. The positive control primer/probe mix can be used with standard TaqMan® cycling conditions and with SYBR® Green Master Mix for detection using SYBR® Green chemistry.

Due to the nature of the cDNA manufacturing process, there may be batch-to-batch potency differences exhibited by the BioBank cDNA. Notwithstanding this, the positive control primers should give a Cq value < 27 when tested on the BioBank cDNA. If this is not achieved, please contact your local Primerdesign representative.

Kit contents

- **BioBank cDNA (250ng, PURPLE)**
- **Control primer/probe mix for 18S ribosomal RNA (BROWN)**
- **RNase/DNase free water (WHITE)**
  for resuspension of primer/probe mixes.
- **Template preparation buffer (YELLOW)**
  for resuspension of BioBank cDNA template.

Reagents and equipment to be supplied by user

- **Real-Time PCR instrument**
- **Precision®PLUS, Precision®FAST or oasig™ 2X qPCR Master Mix**
  This kit is designed to work well with all commercially available master mixes. However, we recommend the use of Primerdesign PrecisionPLUS, PrecisionFAST or oasig 2X qPCR Master Mix.
- **Pipettors and Tips**
- **Vortex and centrifuge**
- **Thin walled 0.2 ml PCR reaction tubes**

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.

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 control

As part of our ISO9001 and ISO13485 quality assurance systems, all Primerdesign products are monitored to ensure the highest levels of performance and reliability.

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Bench-side protocol

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

2. **Resuspend the kit components in RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component	Volume
18S control primer/probe mix (**BROWN**)	50 µl

3. **Resuspend the BioBank cDNA in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex each tube thoroughly.

Component	Volume
BioBank cDNA (**PURPLE**)	130 µl

qPCR

1. **Prepare positive control 18S reactions according to table below:**
   It's recommended to run duplicate wells for your positive control 18s assay.

Component	1 Reaction
PrecisionPLUS, PrecisionFAST or oasig Master Mix	10 µl
18S primer/probe mix (**BROWN**)	1 µl
BioBank cDNA (PURPLE)	5 µl
RNase/DNase free water (WHITE)	4 µl
Final volume	**20 µl**

2. **Prepare target gene reactions according to table below, if using a Primerdesign custom designed qPCR assay (optional):**
   It's recommended to run duplicate wells for your target assay.

Component	1 Reaction
PrecisionPLUS, PrecisionFAST or oasig Master Mix	10 µl
Target gene primer or primer/probe mix	1 µl
BioBank cDNA (PURPLE)	5 µl
RNase/DNase free water (WHITE)	4 µl
Final volume	**20 µl**

3. **Prepare target gene reactions according to table below, if using a user supplied assay (optional):**
   It's recommended to run duplicate wells for your target assay.

Component	1 Reaction
PrecisionPLUS, PrecisionFAST or oasig Master Mix	10 µl
Forward primer	6pmols
Reverse primer	6pmols
Probe (optional)	3pmols
BioBank cDNA (PURPLE)	5 µl
RNase/DNase free water (WHITE)	X µl
Final volume	**20 µl**

qPCR amplification protocol

Please select the correct cycling protocol for the master mix that you are using.

Amplification conditions using PrecisionPLUS or oasig 2X qPCR Master Mix

	Step	Time	Temp
	Enzyme activation	2min	95ºC
X 40 cycles*	Denaturation	10s	95ºC
	DATA COLLECTION**	60s	60ºC

* For low copy number targets, giving late detection, a further 10 cycles may be needed to generate the complete amplification plot.
** Fluorogenic data should be collected during this step through the FAM or SYBR channel, depending on the chemistry being used.

Amplification conditions using PrecisionFAST 2X qPCR Master Mix

	Step	Time	Temp
	Enzyme activation	2min	95ºC
X 40 cycles*	Denaturation	5s	95ºC
	DATA COLLECTION**	20s	60ºC

* For low copy number targets, giving late detection, a further 10 cycles may be needed to generate the complete amplification plot.
** Fluorogenic data should be collected during this step through the FAM or SYBR channel, depending on the chemistry being used.

Please note if using SYBR® Green chemistry a melt curve step should be added to the above amplification conditions after completion of the 40 PCR cycles.