

# EpiQuik™ Quantitative PCR Fast Kit

Base Catalog # P-1029

## PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

**Uses:** The EpiQuik™ Quantitative PCR Fast Kit is designed for quantitative real time analysis of DNA samples from various sources, including DNA immunoprecipitated from CHIP reactions in a fast format.

When using the EpiQuik™ Quantitative PCR Fast Kit for real time PCR, and with very small amounts of input DNA (< 50 pg), the number of PCR cycles should be greater than 45.

Control reactions should be performed to ensure that the PCR primers are specific to the target gene regions. A pair of GAPDH primers specifically for GAPDH promoter regions is included in the kit for determining the efficiency of PCR reactions.

**Precautions:** To lower the risk of contamination during PCR, it is recommended to wear fresh gloves and use pipette tips with aerosol filters during the reaction setup.

## KIT CONTENTS

Component	100 reactions Cat. #P-1029-100	200 reactions Cat. #P-1029-200
Master Mix (2X)	1 vial (1.0 ml each)	2 vials (1.0 ml each)
DNA/RNA-free Water	1 vial (1.2 ml each)	2 vials (1.2 ml each)
<b>Control Primers (GAPDH)</b>		
Forward (100 µM)	8 µl	20 µl
Reverse (100 µM)	8 µl	20 µl

## SHIPPING & STORAGE

The EpiQuik™ Quantitative PCR Fast Kit is shipped on ice packs. The kit should be stored immediately at –20°C upon receipt.

The kit is stable for at least 6 months from the shipment date, when stored properly.

## MATERIALS REQUIRED BUT NOT SUPPLIED

- Real-time PCR instrument
- PCR reaction plates or thin-wall PCR tubes
- Pipette and pipette tips

## GENERAL PRODUCT INFORMATION

**Quality Control:** Each lot of EpiQuik™ Quantitative PCR Fast Kit is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

**Product Warranty:** If this product does not meet your expectations, simply call our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

**Safety:** Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

**Product Updates:** EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at [www.epigentek.com/datasheet](http://www.epigentek.com/datasheet).

**Usage Limitation:** The EpiQuik™ Quantitative PCR Fast Kit is for research use only and is not intended for diagnostic or therapeutic applications.

## A BRIEF OVERVIEW

Quantitative PCR is a useful technique for the analysis of gene expression, gene silencing, protein-DNA interaction, pathogen detection, and various other applications.

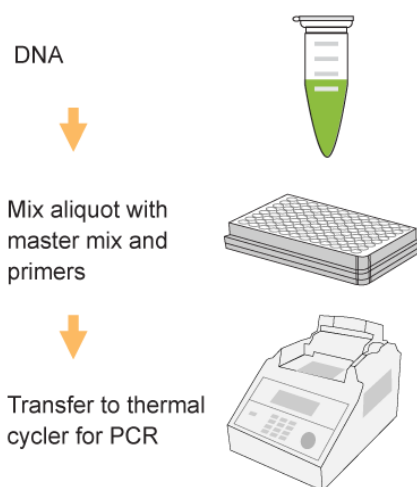
EpigenTek provides the EpiQuik™ Quantitative PCR Fast Kit to allow for a fast, specific, sensitive, and reproducible quantitative real time analysis of DNA samples. The novel hot start DNA polymerase contained in this kit reduces the overall time required for quantitative PCR from approximately 2 hours to just 70 minutes. The kit also facilitates sensitivity and specificity of quantitative PCR by significantly increasing primer-DNA template annealing, while simultaneously reducing non-specific annealing. This results in considerable time savings and a more efficient real time PCR.

The EpiQuik™ Quantitative PCR Fast Kit has the following features:

- An extremely fast procedure that can be finished within 70 minutes.
- Abundant yields due to high amplification efficiency.
- Highly accurate and specific in PCR, which reduces false-positive results.
- Convenient master mix format allows for an easy reaction setup.
- Simple, reliable, and consistent assay conditions.
- Can be used with any block-based real-time PCR instrument

## PRINCIPLE & PROCEDURE

The EpiQuik™ Quantitative PCR Fast Kit provides a master mix format which contains a hot start DNA polymerase, dNTPs, an PCR enhancer, an optimized buffer, and an intercalating green dye. This master mix allows for a convenient and easy reaction setup. The unique hot start DNA polymerase is provided in an inactive state at ambient temperature and is reactivated by several minute long incubations at 95°C, which can easily be integrated into existing thermal cycling steps. The hot start DNA polymerase in combination with the optimized buffer ensures quantitative PCR specificity and sensitivity. The green dye allows for DNA detection and analysis without using a sequence-specific probe.



Schematic procedure for using the EpiQuik™ Quantitative PCR Fast Kit.

## PROTOCOL

### Prepare the PCR Reactions

1. Thaw all reaction components including **Master Mix, DNA/RNA-free Water, Control Primers (GAPDH)**, and DNA template. Mix well by vortexing briefly. Keep components on ice while in use, and return to  $-20^{\circ}\text{C}$  immediately following use.
2. Add components into each well according to the following procedures:

Component	Size ( $\mu\text{l}$ )	Final Concentration
Master Mix (2X)	10 $\mu\text{l}$	1X
Forward Primer	1 $\mu\text{l}$	0.4-0.5 $\mu\text{M}$
Reverse Primer	1 $\mu\text{l}$	0.4-0.5 $\mu\text{M}$
DNA Template	1-2 $\mu\text{l}$	50 pg-0.1 $\mu\text{g}$
DNA/RNA-free H <sub>2</sub> O	6-7 $\mu\text{l}$	
<b>Total Volume</b>	<b>20 <math>\mu\text{l}</math></b>	

**Note:** For the Negative Control, use DNA/RNA-free water instead of DNA template

### Program the PCR Reactions

3. Place the reaction plate in the instrument.
4. Set the PCR conditions as follows:

Cycle Step	Temperature	Time	Cycle
Activation	95°C	60 sec	1
Cycling	95°C 60°C	15 sec 30 sec	40-45
Melt Cure	60-95°C	various	1

## TROUBLESHOOTING

Problem	Possible Causes	Suggestions
Little or No Amplification Product	Primer is incorrect.	Check primer design. If it is specifically for the targeted sequence, confirm the accuracy of the sequence in formation. Redesign the primers according to the guidance of the quantitative PCR primer design.
	Primer is degraded or primer concentration is not optimal.	Repeat PCR with different primer concentrations using 0.1 $\mu\text{M}$ increments. Check for possible degradation of the primers on a denaturing polyacrylamide gel.

	Quality of DNA is poor or input DNA is insufficient.	Check PCR specificity and reliability with GAPDH control primer included in the kit. Increase the amount of input DNA. If necessary, make new DNA sample and repeat PCR with new template.
	DNA is degraded.	Check if DNA is degraded prior to or after DNA modification.
	Incorrect PCR program including insufficient denaturing, annealing & extension time/temperature, and insufficient number of cycles.	Check if the denaturing, annealing, and extension time/temperature programming is correct. Increase number of cycles.
	Hot-start DNA polymerase is not activated.	Perform initial enzyme activation for 7-9 minutes at 95°C.
	Pipetting error or missing reagents.	Check the concentrations and storage conditions of the reagents including primers. Ensure the ratio of master mix to primer-template is 1:1.
	Instrument and PCR plate problem.	Check the power to the real-time PCR instrument and ensure that the PCR plate fits in the instrument.
Non-Specific Amplification Product	Number of PCR cycles is too high.	Reduce the number of cycles to eliminate non-specific PCR.
	Template concentration is too high.	When amplifying DNA, the initial concentration of template in the reaction mixture should not exceed 100 ng per 20 µl of reaction volume.
	Primer design is not optimal.	Check primer design and confirm the accuracy of the sequence in formation. Redesign the primers according to the guidance of the PCR primer design or by using MethPrimer.

## RELATED PRODUCTS

### Chromatin Preparation

P-2001 ChromaFlash™ Chromatin Extraction Kit

### DNA Preparation and Cleanup

P-1003 FitAmp™ General Tissue Section DNA Isolation Kit  
P-1004 FitAmp™ Plasma/Serum DNA Isolation Kit  
P-1006 DNA Concentrator Kit  
P-1009 FitAmp™ Paraffin Tissue Section DNA Isolation Kit  
P-1017 FitAmp™ Urine DNA Isolation Kit  
P-1018 FitAmp™ Blood and Cultured Cell DNA Extraction Kit

### Chromatin Immunoprecipitation

P-2025 ChromaFlash™ One-Step ChIP Kit  
P-2026 ChromaFlash™ One-Step Magnetic ChIP Kit