

Probes for Quantitative, Real-Time PCR

Operon® offers high-quality probes for quantitative, real-time PCR. These probes can be used to detect sequence differences for mutation detection and allele discrimination. Operon probes for quantitative, real-time PCR are based on oligos labeled with fluorescent dyes and quenchers. A wide variety of fluorophores and quenchers are available.

Introduction

Dual-labeled probes have a fluorescent reporter and a quencher at their 5' and 3' ends, respectively. These probes can be used in quantitative PCR systems that take advantage of the 5'-3' exonuclease activity of Taq DNA polymerase. A probe specific for the sequence of interest is used in PCR together with specific PCR primers. This probe is designed to anneal between the PCR primers. During the extension phase of PCR, the 5'-3' exonuclease activity of Taq DNA polymerase cleaves the fluorescent reporter from the probe. The amount of free reporter accumulates as the number of PCR cycles increases. The fluorescent signal from the free reporter is measured in real time and allows quantification of the amount of target sequence.

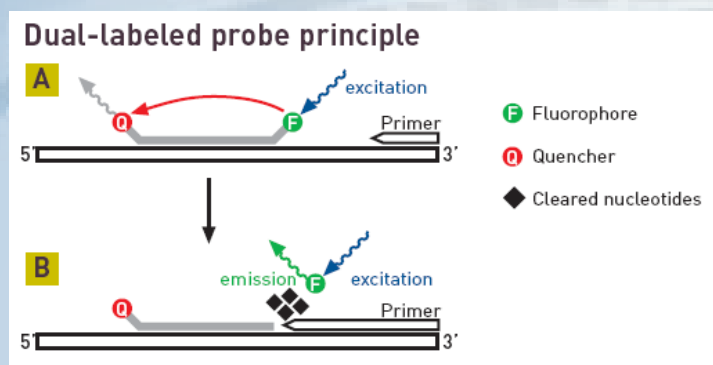


Figure 1. Schematic diagram of the principle of dual-labeled probes in quantitative, real-time PCR.

A) Both the dual-labeled probe and the PCR primers anneal to the target sequence during the PCR annealing step. The proximity of the fluorescent reporter with the quencher prevents the reporter from fluorescing.
 B) During the PCR extension step, Taq DNA polymerase extends the primer. When the enzyme reaches the dual-labeled probe, its 5'-3' exonuclease activity cleaves the fluorescent reporter from the probe. The fluorescent signal from the free reporter is measured.

Black Hole Quenchers

Black Hole Quenchers (BHQs) are a novel class of high-efficiency dark quenchers that prevent fluorescence until a hybridization event occurs. The BHQ family of quenchers was developed to provide excellent spectral overlap over the entire range of commonly used reporter dyes. Therefore, the BHQs are

optimal for multiplexing approaches. They can be used as quenchers for standard dual labeled probes or for Molecular Beacons.

3' Modification	5' Modification
TAMRA	Fluorescein, 6-FAM, HEX, TET
Black-Hole Quencher	Fluorescein, 6-FAM, TET, HEX, Cy3, Cy3.5, Cy5, Cy5.5, TAMRA, ROC, Texas Red, Oregon Green, JOE, Bodipy TMR

Table 1. Commonly used dual-labeled probes

Benefits of BHQs include:

- o No native fluorescence
- o Optimized signals
- o Minimized background noise
- o Superior spectral overlap

More dyes and quenchers are available, such as Cascade Blue, Alexa Fluor dyes, Bodipy dyes, and QSY dark quenchers. All dyes and quenchers can be freely combined according to your needs.

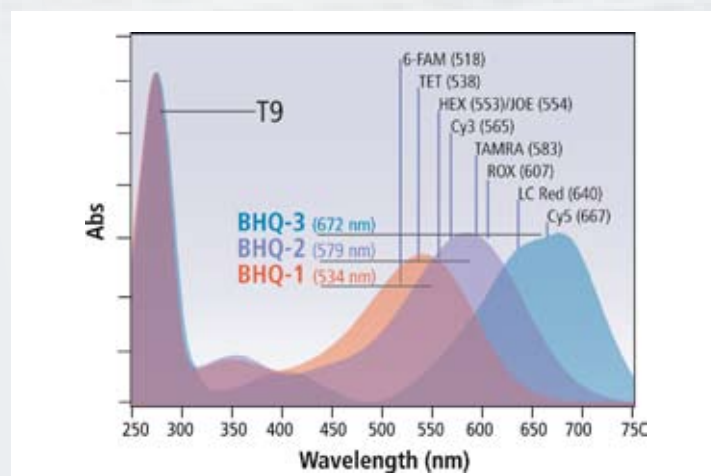


Figure 2. Absorption spectra
 Absorption spectra of the three BHQ dyes (conjugated to T-9 and normalized to the poly-T absorbance of 260 nm) with the emission maxima of many commonly used reporter groups indicated. With permission of Biosearch Technologies, Inc.