

www.genXpress.at - Amplifluor™ Apoptosis Gene Systems

Genes such as *fas*, *bax-alpha* and *bcl-X_L* are differentially expressed during apoptosis. *Fas* protein binds to the *fas* receptor inducing apoptosis in target cells such as cytotoxic T cells in cancer cells. *Bax*, *bcl-2* and *bcl-X_L* belong to a family of cytoplasmic proteins functioning as either pro-apoptotic (*bax*) or anti-apoptotic (*bcl-2* and *bcl-X_L*) regulators of apoptosis. The ratio of expression between *bax*: *bcl-2* or *bax*: *bcl-X_L* determines whether cells undergo apoptosis or survive. Elevated expression of *bcl-2* and *bcl-X_L* contributes to cancer development. Quantitative PCR (QPCR) is an extremely powerful method of measuring the expression of these apoptotic genes at the mRNA level. Amplifluor™ Apoptosis Gene Systems enable you to measure the expression of *fas*, *bax-alpha*, *bcl-2* and *bcl-X_L* mRNA with precision using fluorescence QPCR technology. Amplifluor™ QPCR technology is a rapid and more precise alternative to cumbersome gene expression methods such as Northern blotting and RT PCR gel-based assays. Results are obtained during the PCR (real-time) or at a single point during the exponential amplification range (endpoint).

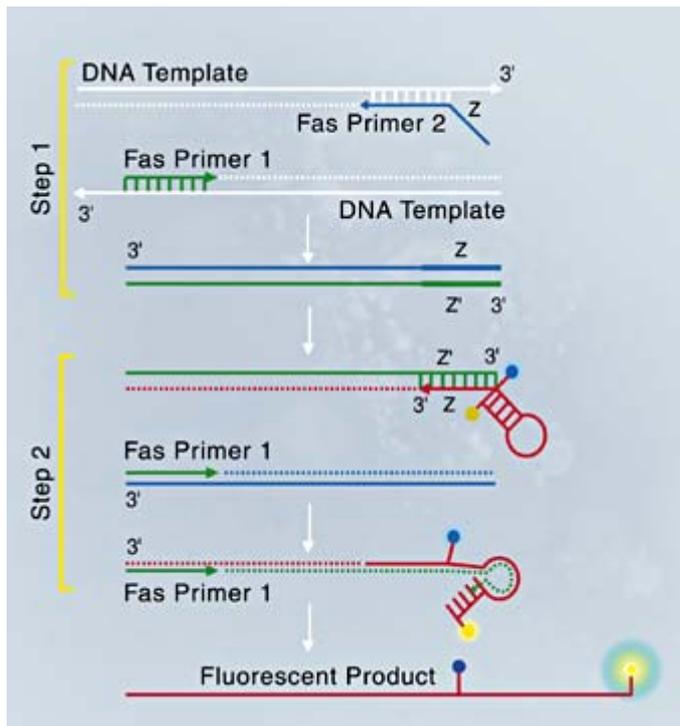
Features

- Non-gel approach saves time and permits the rapid analysis of a large amount of samples
- Cost-effective quantitative PCR system
- All systems utilize the same PCR conditions permitting multi-gene analysis of a single sample in one PCR run
- Includes all primers and quantitation controls: simply provide the experimental cDNAs and licensed PCR reagents
- Measure results using practically any fluorescence measuring instrument including real-time instruments and fluorescence plate readers
- Sensitivity and linearity:
 - &SHY; Real-time: 100 copy sensitivity with 7 orders of linear dynamic range
 - &SHY; Fluorescence plate readers: 1000 copy sensitivity with 3 orders of linear dynamic range

Principles of Amplifluor™ Apoptosis Gene Systems

Typical methods of mRNA quantitation involve competitive PCR assays that require gel analysis and other post-PCR processing. Amplifluor™ technology offers a non-gel approach to QPCR using real time and single point analysis. Using Amplifluor™ technology, PCR and fluorescence measurements are performed simultaneously. Amplifluor™ Apoptosis Gene Systems utilize QPCR with energy transfer-labeled primers. Each system contains 3 oligonucleotide primers and a quantitation control. Two of the primers function to specifically amplify the target. The third primer is an energy-transfer labeled primer that generates fluorescence when it is specifically incorporated into PCR. This third primer is the [UniPrimer™ Energy Transfer-labeled Primer](#).

Using Amplifluor™ Apoptosis Gene Systems is easy and requires only the ability to perform routine PCR. Amplifluor™ Apoptosis Gene Systems primers, Taq Polymerase, 10X buffer and dNTPs and the experimental cDNA sample are combined in a PCR tube and subjected to PCR. During the PCR specific amplification of the target apoptosis gene of interest occurs producing fluorescence. The amount of fluorescence is directly proportional to the accumulation of PCR product at each cycle. The following example illustrates the PCR using the Amplifluor™ *fas* Gene System:



Principle of the Amplifluor™ *fas* Gene System: the *fas* target-specific primer pair (1 and 2) amplifies the desired cDNA target. Fluorescence signal is generated when UniPrimer™ is specifically incorporated into the amplicon via its "Z sequence". *Fas* primer 1 is an untailed primer while the *fas* primer 2 contains a single-stranded "Z sequence" tail at its 5' end. The "Z sequence" of primer 2 is identical to the "Z sequence" of the UniPrimer™. In step 1 of the reaction, the extension of the tailed *fas* primer (primer 2) yields a product that contains the "Z sequence". When *fas* primer 1 anneals to this template and is extended, the product contains the complement of the Z sequence (Z' sequence). In step 2 of the reaction, UniPrimer™ anneals to the template containing the Z' sequence. During the polymerization reaction the fluorescence reporter (o) and quencher (OO) molecules are incorporated into the product. This PCR product serves as a template for *fas* primer 1. As the *fas* primer 1 is extended the hairpin conformation of the template is unfolded. The fluorescein and DABSYL are no longer physically close enough to permit quenching, and a fluorescence signal is emitted. The UniPrimer™ and *fas* primer 1 are used in equimolar concentrations and the *fas* primer 2 concentration is 10X less.

The unique design of UniPrimer™ is such that it emits a fluorescence signal only upon its incorporation into the amplification products produced during each PCR cycle.

Unincorporated UniPrimer™ does not fluoresce which eliminates the need to purify the amplification products prior to quantitation. Quantitation can therefore be performed during the PCR reaction (real time) or at a single point taken in the exponential amplification stage of the reaction (endpoint).

Instrument Options

Amplifluor™ technology is uniquely compatible with virtually any instrument that measures fluorescence.

Real-time Detection Instruments:

Real-time detection instruments measure fluorescence accumulation at each cycle of PCR. When using a real-time measuring device with UniPrimer™ reactions, program the instrument to measure fluorescence during the annealing step at temperatures between 50-60°C. At this temperature, unincorporated UniPrimer™ is in the silent hairpin conformation and will not contribute to fluorescence measurements.

Amplifluor™ technology has been used successfully on the ABI PRISM™ 7700, the LightCycler™, and the iCycler™.

Endpoint Detection Instruments:

Fluorescence Plate Reader:

In this option, PCR is performed using any temperature cycler then read directly on a fluorescence plate reader without having to transfer the samples from the PCR vessel to another plate format, such as a microtiter plate. Amplifluor™ Systems are optimized for maximum sensitivity in the fluorescence plate reader format. The instruction manuals specify cycling and reaction conditions for single point readings taken at the end of the reaction (endpoint). These single point determinations are made in the exponential amplification range of the PCR. Advantages of this method are:

- Closed-tube method of amplification and detection
- Direct sample readout facilitates quantification
- Advanced plate design facilitates higher throughput than many real-time instruments

Spectrofluorometer:

The yield of the PCR reaction may be determined by placing a diluted aliquot of each completed reaction in a cuvette and measuring the fluorescence in a spectrofluorometer. To read fluorescence, set the excitation setting to 495 nm and the emission setting to 516 nm.

UV transilluminator/camera:

Amplifluor™ reactions are easily visualized using this method. PCR is performed in standard reaction vessels (tubes, tube strips or plates) or in the optional gel format. Because the amplicon is directly labeled with fluorescence, no ethidium bromide is required for visualization. (Please inquire regarding the use of filters with your standard camera or CCD-based gel imaging system). Though not as quantitative as other instrument options, this method provides a rapid, "yes" or "no" answer for researchers wanting to confirm the success of their amplification reactions. Additionally, this option is useful when constructing gene-specific primers required for the Amplifluor™ Universal Amplification and Detection System (#S7901).

Fluoroimaging gel systems:

This method is used similarly to the method above but is a more quantitative method. We have used the FMBIO® II successfully for several Amplifluor™ gel-based analysis assays.

Amplifluor™ Gene Systems Components

All Amplifluor™ Gene Systems include UniPrimer™, target-specific primers and a control template. Buffer, polymerase and dNTPs are purchased separately.

	Amplifluor™ UniPrimer™ (fluorescein) GX-30-S7901 100 rct GX-30-S7905 1000 rct GX-30-S7903 10.000 rct	10X Tailed Primer	10X Untailed Primer	Control Template
Amplifluor™ <i>bax-alpha</i> Gene System, Human GX-30-S7913 100 rct		<i>bax-alpha</i> (40-mer)	<i>bax-alpha</i> (22-mer)	
Amplifluor™ <i>bcl-2</i> Gene System, Human GX-30-S7914 100 rct		<i>bcl-2</i> (37-mer)	<i>bcl-2</i> (24-mer)	
Amplifluor™ <i>bcl-X</i> (long) Gene System, Human GX-30-S7915 100 rct		<i>bcl-X</i> (long) (39-mer)	<i>bcl-X</i> (long) (22-mer)	
Amplifluor™ <i>fas</i> Gene System, Human GX-30-S7916 100 rct		<i>fas</i> (41-mer)	<i>fas</i> (23-mer)	
Amplifluor™ HPV-16 Gene System, Human GX-30-100 rct		HPV-16 (40-mer)	HPV-16 (24-mer)	
Amplifluor™ PSA Gene System, Human GX-30-S7911 100 rct		PSA (36-mer)	PSA (24-mer)	