

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



# Universal ProbeLibrary Extension Set, Probes #91 to #165

 **Version: 07**

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Set of 75 short hydrolysis probes for gene expression quantification of multiple organism levels by real-time PCR

**Cat. No. 04 869 877 001**    1 set  
75 Universal ProbeLibrary probes, 125 µl, 10 µM, each.

**Store the set at –15 to –25°C.**

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# 1. General Information

## 1.1. Contents

Cap	Label	Function / Description	Content
colorless	“Universal ProbeLibrary, #91” to “Universal ProbeLibrary, #165”	75 vials, each vial contains a 10 µM solution of a pretested probe.	75 vials, 125 µl each

- i** When ordered in combination with the Universal ProbeLibrary Set, Human, which contains Universal ProbeLibrary probes #1 to #90, the whole set of the 165 Universal ProbeLibrary probes is obtained.
- i** You can order each probe of the Universal ProbeLibrary separately as a single probe package. Each Universal ProbeLibrary Probe package contains 2 vials, each vial containing 125 µl of a 10 µM probe solution.

## 1.2. Storage and Stability

### Storage Conditions (Product)

The set is shipped at ambient temperature.

When stored at –15 to –25°C, the set is stable through the expiration date printed on the label.

Cap	Label	Storage
colorless	“Universal ProbeLibrary, #91” to “Universal ProbeLibrary, #165”	Store at –15 to –25°C. <b>⚠ Keep protected from light.</b> <b>⚠ Avoid repeated freezing and thawing (more than 5 times).</b> <b>After the first thaw, aliquot each probe, then store.</b> For short-term storage for up to 1 month, the probes may be stored at +2 to +8°C.

## 1.3. Additional Equipment and Reagents Required

### Standard Laboratory Equipment

- Nuclease-free pipette tips
- 1.5 ml RNase-free microcentrifuge tubes to prepare master mixes and dilutions.
- To minimize risk of RNase contamination, autoclave all vessels.

### For First Strand cDNA Synthesis

- Transcriptor First Strand cDNA Synthesis Kit\*

### For use in Combination with the LightCycler® 480 System

- LightCycler® 480 Instrument\*
- LightCycler® 480 Probes Master\*
- LightCycler® 480 Multiwell Plate 96\* or 384\*
- LightCycler® 480 Sealing Foil\*
- Standard swinging-bucket centrifuge containing a rotor and adapters for multiwell plates

### For use in Combination with the LightCycler® Carousel-Based System

- LightCycler® 2.0 Instrument, or LightCycler® 1.5 Instrument, or instrument version below
- LightCycler® TaqMan® Master\*
- LightCycler® Capillaries\*
- LC Carousel Centrifuge 2.0\* (optional)

## 1. General Information

### For use in Combination with Other Real-Time PCR Instruments

- FastStart Universal Probe Master (Rox)\* (for use with instruments requiring a reference dye)
- FastStart TaqMan® Probe Master\* (for use with instruments not requiring a reference dye)

### For PCR Product Carryover Prevention (optional)

- LightCycler® Uracil-DNA Glycosylase\* (for use with all LightCycler® kits and reagents using the FastStart enzyme)
- Uracil-DNA Glycosylase, heat-labile\* (for use with other real-time PCR reagents)

## 1.4. Application

The Universal ProbeLibrary Extension Set is intended for life science research. In combination with the Universal ProbeLibrary Set, Human (containing Universal ProbeLibrary Probes #1 to #90), it enables rapid and flexible quantification of virtually any transcript in the transcriptomes of a large number of organisms by real-time PCR assays. Performance of the assay with the selected Universal ProbeLibrary probe follows established real-time PCR protocols using the LightCycler® TaqMan® Master on the LightCycler® Carousel-Based System, or the LightCycler® 480 Probes Master on the LightCycler® 480 System, or the FastStart TaqMan® Probe Master (or other real-time PCR compatible reagents) on other real-time PCR instruments. Universal ProbeLibrary probes are labeled with fluorescein (FAM) at the 5' end and with a dark quencher dye near the 3' end. The probes can be detected using standard FAM or SYBR Green I filters.

Gene-specific expression-quantification assays are easily designed using the web-based ProbeFinder software at [www.universalprobelibrary.com](http://www.universalprobelibrary.com).

## 1.5. Preparation Time

### Assay Time

A Universal ProbeLibrary assay includes assay design, ordering primers, and the real-time PCR reaction. The assay takes approximately 2 days.

The steps below describes the basic workflow of a Universal ProbeLibrary assay:

#### Day 1

Decide on target sequence of interest, for example, entire transcript, exon, or other sequence.

Identify probes and primers using the ProbeFinder software.

Order primers from your preferred oligo supplier for overnight delivery.

**i** *If you want to order a single Universal ProbeLibrary probe based on the ProbeFinder result, you can directly switch to the Online Ordering site from the ProbeFinder software. Simply submit the probe name or the catalog number found on the ProbeFinder result screen. In this case, extra delivery time for the probe should be considered.*

#### Day 2

Combine the relevant probe with the corresponding primers.

Perform real-time PCR.

**i** *Depending on the LightCycler® System you are using, the run times vary between 50 to 70 minutes.*

Evaluate results.

## 2. How to Use this Product

### 2.1. Before you Begin

#### Sample Materials

#### Amplification of cDNA Using the Universal ProbeLibrary Assays

##### RNA Preparation

Before setting up a Universal ProbeLibrary assay, convert RNA to cDNA using a reverse transcription reaction. To obtain accurate and reproducible results, use a high quality RNA preparation method for reverse transcription. Total RNA should be non-degraded and free of contaminating DNA, RNases, and inhibitors. For reproducible isolation of nucleic acids, we recommend:

- Either a MagNA Pure System together with a dedicated reagent kit (for automated isolation),
- or a High Pure Nucleic Acid Isolation Kit (for manual isolation).

Total RNA quality can be verified using gel electrophoresis to show that it is non-degraded. An  $OD_{260/280}$  measurement should also be performed; high quality RNA has an  $OD_{260/280}$  ratio of 1.8 to 2.0.

As a general guideline, a cDNA concentration of 5 ng equivalent of total RNA/PCR reaction is sufficient for detecting medium abundant mRNA. The optimal RNA amount is dependent on the abundance of your transcript. For less abundant transcripts, a higher amount of total RNA is required. To avoid DNA contamination, we recommend using a DNase-treated RNA preparation. Whenever non-intron-spanning assays are performed, the use of DNase-treated RNA preparations is mandatory.

##### cDNA Preparation

Preparing high quality first strand cDNA is essential for real-time PCR. Reverse transcription of RNA can be primed using random hexamer, oligo(dT) or gene-specific primers. Because the positions of the genes in UPL assays can be throughout the entire length of transcripts, Roche recommends using a combination of random hexamer and oligo(dT) priming to avoid 3' bias in the cDNAs. Since reverse transcription is an essential step for obtaining good results, Roche recommends using the Transcriptor First Strand cDNA Synthesis Kit to prepare your cDNA.

**⚠ To minimize the risk of PCR inhibition, the input volume of sample cDNA for the PCR should not exceed 5% of the total reaction volume. Roche recommends diluting the cDNA at least 1:5 for subsequent input into a single reaction. For initial experiments, run undiluted, 1:10 diluted, and 1:100 diluted cDNA template in parallel to determine the optimal template amount.**

### Control Reactions

#### Negative Controls

Always run negative controls with the samples. To prepare negative controls:

- Replace the template cDNA with PCR-grade water to reveal contamination problems (no template control).
- Omit addition of reverse transcriptase to the cDNA synthesis reaction to indicate false positive results due to the presence of DNA in the RNA sample (RT-minus control). Alternatively, untranscribed sample RNA can be used in PCR as an RT-minus control. In the PCR reaction, use an RNA sample amount that is equivalent to the cDNA sample to be tested.

## 2. How to Use this Product

### Primers

Suitable concentrations of primers range from 200 to 900 nM (final concentration). The recommended starting concentration is 400 nM each. The primer concentration may be optimized by varying the final concentration from 200 to 900 nM in increments of 100 nM.

**⚠ To assure optimum performance of your Universal ProbeLibrary assay, always use highly purified (e.g., HPLC purified) PCR primers. Optimize the primer concentration first, then determine the probe optimization using the optimized primer concentrations.**

**i** The optimal probe concentration is the lowest concentration that results in the lowest C<sub>q</sub> (or C<sub>p</sub>) and an adequate fluorescence for a given target concentration.

### Probe

Suitable concentrations of Universal ProbeLibrary probes range from 100 to 200 nM (final concentration). Typically, a probe concentration of 100 nM results in a lower fluorescence signal intensity than a probe concentration of 200 nM. This does not usually influence the performance of the assay. The Universal ProbeLibrary probe concentration may be optimized by varying the final concentration from 100 to 500 nM in increments of 100 nM.

**i** The optimal probe concentration is the lowest concentration that results in the lowest C<sub>q</sub> (or C<sub>p</sub>) and an adequate fluorescence for a given target concentration.

## General Considerations

### Precautions

To reduce the risk of contaminating your PCR reaction with PCR amplicons generated in previous reactions (and consequently false results), as well as of degrading template nucleic acid, follow the recommendations below:

- Always wear a clean lab coat. Use separate lab coats when setting up PCR reaction and handling PCR products.
- Change gloves whenever you suspect they have been contaminated.
- Maintain dedicated areas for PCR setup, PCR amplification, and gel electrophoresis of PCR products.
- Always use nuclease-free reagents, buffers, and consumables.
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Pipette carefully and do not splash or spray PCR samples.
- Keep reactions and components capped whenever possible. Always spin tubes before opening.
- Use pipette tips with aerosol filter inserts to avoid aerosol-mediated contamination of your pipetting device.
- Clean laboratory benches and equipment regularly.
- Prevent carryover contamination by using dUTP and Uracil-DNA Glycosylase.

### Number of Tests

The number of tests that can be performed will depend on the final concentration of the UPL probe in your assay and on the reaction volume. The Universal ProbeLibrary probe concentration may be optimized by varying the final concentration from 100 to 500 nM. When using the probe at a final concentration of 200 nM, each vial contains enough probe for 312 assays in a 20 µl real-time PCR setup.

### Passive Reference Dye

The LightCycler® Instruments do not require addition of a passive reference dye to the PCR reaction. Other real-time PCR instruments may require addition of a passive reference dye, usually Rox. The reference dye is used to normalize signals from individual reaction wells to compare real-time PCR amplification signals across an entire PCR plate. The amount of Rox in the PCR reaction will depend on the real-time PCR instrument and must be adjusted accordingly.

**i** Refer to the Operator's Manual of your real-time PCR instrument for additional information.

## 2.2. Protocols

### LightCycler® 480 Instrument Protocols

The following protocol is optimized for use with the LightCycler® 480 System.

**⚠ Program the LightCycler® 480 Instrument before preparing the reaction mixes.**

A LightCycler® 480 Instrument protocol that uses the LightCycler® 480 Probes Master contains the following programs:

- **Pre-Incubation** for activation of FastStart Taq DNA Polymerase and denaturation of template cDNA
- **Amplification** of target DNA
- **Cooling** the plate and thermal block cycler

**i** For details on how to program the experimental protocol, see the LightCycler® 480 Operator's Manual.

### Protocol for Use with the LightCycler® 480 Instruments I and II (Multiwell Plate 96 or 384)

The following table shows the parameters that must be programmed for a PCR run using the LightCycler® 480 Probes Master.

Setup					
Block Type		Reaction Volume [μl]			
96 (384)		20 (10)			
Detection Format		Excitation Filter		Emission Filter	
Mono Color Hydrolysis Probe / UPL Probe					
LightCycler® 480 Instrument I: FAM		483		533	
LightCycler® 480 Instrument II: FAM		465		510	
Program Name		Cycles		Analysis Mode	
Pre-Incubation		1		None	
Amplification		45		Quantification	
Cooling		1		None	
Temperature Targets					
	Target [°C]	Acquisition Mode	Hold [hh:mm:ss]	Ramp Rate [°C/s]	Acquisitions [per °C]
Pre-Incubation	95	None	00:10:00	4.4 (4.8)	–
Amplification	95	None	00:00:10	4.4 (4.8)	–
	60	None	00:00:30	2.2 (2.5)	–
	72	Single	00:00:01	4.4 (4.8)	–
Cooling	40	None	00:00:30	2.2 (2.5)	–

## 2. How to Use this Product

### Preparation of the PCR Mix

Use the LightCycler® 480 Probes Master to perform UPL assays on the LightCycler® 480 Instruments.

Follow the protocol below to prepare one 20 µl reaction using the LightCycler® 480 Probes Master.

**⚠ Do not touch the surface of the LightCycler® 480 Multiwell Plate when handling the plate. Always wear gloves.**

- 1 Thaw the solutions and briefly spin vials in a microcentrifuge before opening.
  - Mix carefully by pipetting up and down and store on ice.

- 2 Prepare the PCR mix in a suitably sized tube on ice. The total volume will depend on the number of samples.

**⚠ When setting up the PCR mix, compensate for pipetting losses. Roche recommends preparing the PCR mix with 10% overdosage (one extra sample for every 10).**

– Prepare the PCR Mix for one 20 µl reaction by adding the following components in the order listed below:

Reagent	Conc. [µM]	Volume [µl]	Final conc. [nM]
LightCycler® 480 Probes Master, Water, PCR Grade (Vial 2)	–	3.8	–
Primer (GOI) forward	20.0	0.4	400
Primer (GOI) reverse	20.0	0.4	400
UPL Probe (GOI)	10.0	0.4	200
LightCycler® 480 Probes Master, (Vial 1)	2x	10.0	1x
<b>Total Volume</b>		<b>15.0</b>	

- 3 Mix carefully by pipetting up and down. Do not vortex.

- 4 Pipette 15 µl of the PCR mix into each well of the LightCycler® 480 Multiwell Plate.
  - Pipette 5 µl of template (cDNA) into the PCR mix of each well.

- 5 Seal the Multiwell Plate with a LightCycler® 480 Sealing Foil.

- 6 Centrifuge the Multiwell Plate for 2 minutes at 1,500 × *g* in a standard swinging-bucket centrifuge using a rotor for multiwell plates and suitable adaptors.
  - Be sure to balance the plate with a suitable counterweight (e.g., another Multiwell Plate).

- 7 Transfer the Multiwell Plate into the plate holder of the LightCycler® 480 Instrument.

- 8 Start the LightCycler® 480 Instrument run by using the PCR program as described above.



## Protocol for Use with the LightCycler® Carousel-Based Systems

The following protocol is optimized for use with the LightCycler® Carousel-Based System.

**⚠ Program the LightCycler® Instrument before preparing the reaction mixes.**

A LightCycler® Carousel-Based Instrument protocol that uses the LightCycler® TaqMan® Master contains the following programs:

- **Pre-incubation** for activation of FastStart Taq DNA Polymerase and denaturation of the cDNA
- **Amplification** of the target DNA
- **Cooling** of the rotor and thermal chamber

**i** For details on how to program the experimental protocol, see the LightCycler® Instrument Operator's Manual.

The following table shows the PCR parameters that must be programmed for a LightCycler® Carousel-Based system PCR run with the LightCycler® TaqMan® Master.

### LightCycler® Software Version 4.1

Programs				
Setup	Setting			
Default Channel	Fluorescence Channel 530			
Seek Temperature	30°C			
Max Seek Pos.	Enter the total number of sample positions the instrument should look for.			
Instrument Type	"6 Ch." for LightCycler® 2.0 Instrument or "3 Ch." for LightCycler® 1.5 Instrument			
Capillary Size	Select "20 µl" as the capillary size for the experiment.			
Programs				
Program Name	Cycles	Analysis Mode		
Pre-Incubation	1	None		
Amplification	45	Quantification		
Cooling	1	None		
Temperature Targets				
	Target [°C]	Hold [hh:mm:ss]	Ramp Rate [°C/s]	Acquisition Mode [per °C]
Pre-Incubation	95	00:10:00	20	None
Amplification	95	00:00:10	20	None
	60	00:00:30	20	None
	72	00:00:01	20	Single
Cooling	40	00:00:30	20	None

## 2. How to Use this Product

### Preparation of the Master Mix

Use the LightCycler® TaqMan® Master to perform UPL assays on the LightCycler® Carousel-Based Instruments. Prepare the 5x Master Mix of the LightCycler® TaqMan® Master as described below:

1 Thaw one vial of Reaction Mix (Vial 1b).

2 Briefly centrifuge one Vial 1a (Enzyme) and one thawed Vial 1b (Reaction Mix from Step 1).

3 Pipette 10 µl from Vial 1a into Vial 1b.

*i Each Vial 1a contains enough enzyme for three vials of Reaction Mix.*

4 Mix gently by pipetting up and down. Do not vortex.

5 Re-label Vial 1b with the new label (Vial 1: Master Mix) provided with the kit.

*i The volume of the resulting Master Mix (5x conc.) is sufficient for 32 reactions with a final reaction volume of 20 µl.*

### Preparation of the PCR Mix

Follow the procedure below to prepare one 20 µl reaction using the LightCycler® TaqMan® Master.

**⚠ Do not touch the surface of the capillaries. Always wear gloves when handling the capillaries.**

1 Thaw the solutions and briefly spin vials in a microcentrifuge before opening.  
– Mix carefully by pipetting up and down and store on ice.

2 Prepare the PCR mix in a suitably sized tube on ice. Total volume will depend on the number of samples.

**⚠ When setting up the PCR mix, compensate for pipetting losses. Roche recommends preparing PCR mixes with 10% overdosage (one extra sample for every 10).**

– Prepare the PCR Mix for one 20 µl reaction by adding the following components in the order mentioned below:

Reagent	Conc. [µM]	Volume [µl]	Final conc. [nM]
LightCycler® TaqMan® Master, Water, PCR Grade (Vial 2)	–	9.8	–
Primer (GOI) forward	20.0	0.4	400
Primer (GOI) reverse	20.0	0.4	400
UPL Probe (GOI)	10.0	0.4	200
LightCycler® TaqMan® Master, Master Mix (Vial 1)	5x	4.0	1x
<b>Total Volume</b>		<b>15.0</b>	

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- 3 Mix carefully by pipetting up and down. Do not vortex.

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  - 4 Pipette 15 µl of the PCR mix into each LightCycler® Capillary.
    - Add 5 µl of the cDNA template.

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  - 5 Seal each capillary with a stopper.

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  - 6 Centrifuge the LightCycler® Sample Carousel containing the capillaries in the LC Carousel Centrifuge.
    - Alternatively, place the adapters containing the capillaries into a standard benchtop microcentrifuge.
    - Centrifuge at  $700 \times g$  for 5 seconds (3,000 rpm in a standard benchtop microcentrifuge).
    - Transfer the capillaries into the sample carousel of the LightCycler® Instrument.

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  - 7 Start the LightCycler® Instrument run by using the PCR program as described above.
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## Protocol for Use with Other Real-Time PCR Instruments

Universal ProbeLibrary assays are compatible with all real-time PCR instruments capable of detecting fluorescein, FITC, FAM, and/or SYBR Green I. Use a standard real-time PCR protocol for hydrolysis probes, taking into account recommendations in the supplier's pack insert of each respective master mix.

*i* For details on how to program the experimental protocol, refer to the Operator's Manual of your real-time PCR instrument.

**⚠ For best results, be sure the instrument is correctly calibrated. Set the detection channel of your real-time PCR instrument to either SYBR Green I or FAM (i.e., 530 nm) for detection of UPL probes labeled with FAM. For the Universal ProbeLibrary reference gene assays, use the VIC/HEX channel (or the next possible emission filter moving to longer wavelengths, for example, 560 nm or 568 nm).**

*i* When performing UPL assays on Applied Biosystems' Fast Real-time PCR Systems, the use of the Fast Mode protocol may generate sub-optimal results.

## 2. How to Use this Product

### Preparation of the PCR Mix

Depending on the real-time PCR instrument you use, PCR reagents containing a reference dye (Rox) may be required. Roche recommends using the FastStart Universal Probe Master (Rox) with instruments requiring a reference dye, and the FastStart TaqMan® Probe Master with instruments not requiring a reference dye. For detailed information on how to use the FastStart Universal Probe Master (Rox) or the FastStart TaqMan® Probe Master, refer to the corresponding Instructions for Use.

Follow the procedure below to prepare one 20 µl reaction.

- 1 Thaw the solutions and, to ensure recovery of all the contents, briefly spin vials in a microcentrifuge before opening.
  - Mix carefully by pipetting up and down and store on ice.

- 2 Prepare the PCR mix in a suitably sized tube on ice. The total volume will depend on the number of samples.

**⚠ When setting up the PCR mix, compensate for pipetting losses. We recommend preparing the PCR mix with 10% overdosage (one extra sample for every 10).**

- Prepare the PCR mix for one 20 µl reaction mix by adding the following components in the order listed below:

Reagent	Conc. [µM]	Volume [µl]	Final conc. [nM]
Water, PCR Grade	-	3.8	-
Primer (GOI) forward	20.0	0.4	400
Primer (GOI) reverse	20.0	0.4	400
UPL Probe (GOI)	10.0	0.4	200
FastStart Universal Probe Master (Rox) or Fast Start TaqMan® Probe Master	2x	10.0	1x
<b>Total Volume</b>		<b>15.0</b>	

- 3 Mix carefully by pipetting up and down. Do not vortex.

- 4 Pipette 15 µl of the PCR mix into each reaction device of your real-time PCR system.
  - Pipette 5 µl of template (cDNA) to the PCR mix into each reaction device.

- 5 Continue according to the instructions of your real-time PCR system.

## 3. Troubleshooting

Observation	Possible cause	Recommendation
No positive fluorescent signal during real-time PCR.	Insufficient template amount.	Add more cDNA when working with very low abundance transcripts. Include more RNA in your reverse transcription reaction.
	Transcript not present in sample material.	
	Incomplete reverse transcription.	Use a combination of oligo(dT) and random hexamer for priming of the reverse transcription reaction.
	PCR inhibition	The amount of sample cDNA pipetted into the PCR reaction should not exceed 5% of the total reaction volume. Therefore, Roche recommends diluting the cDNA at least 1:5 fold for a single PCR reaction.
Log-linear phase of amplification just begins at the end of the cycling program.	Wrong detection channels or parameters.	Check your PCR protocol.
	Number of cycles in the PCR protocol is too low.	Run a PCR protocol with 45 to 50 cycles.
RT-minus control with positive signal.	Insufficient template amount.	Add more cDNA when working with very low abundance transcripts. Include more RNA in your reverse transcription reaction.
	Amplification of remaining genomic DNA in the RNA sample preparation.	Improve your RNA preparation, for example, with a DNase treatment. Check your UPL assay design. For cDNA amplification, the design of an intron-spanning assay helps to avoid false positive signals due to contaminating genomic DNA. When using the option "Automatically select an intron-spanning assay", UPL assays that are designed by ProbeFinder target an intron-spanning site.
Negative control sample with positive signal.	Contamination	Use Uracil-DNA Glycosylase for PCR product carryover prevention.
		Check your PCR setup workflow.

## 4. Additional Information on this Product

### 4.1. Test Principle

The Universal ProbelLibrary is a powerful system for quantifying the expression level of virtually any transcript of a given organism using real-time qPCR assays. It comprises 165 pretested, real-time PCR detection probes and ProbeFinder Software for the design of gene-specific assays.

Universal ProbelLibrary (UPL) probes are labeled at the 5' end with fluorescein (FAM) and at or near the 3' end with a quencher dye. The extensive transcript coverage of UPL probes is due to their short length of just 8 to 9 nucleotides of carefully selected sequences. In order to maintain the hybridization stability and specificity required for qPCR probes, Locked Nucleic Acids (LNAs) are incorporated into the sequence of each UPL probe. LNAs are DNA nucleotide analogs with increased binding strengths compared to standard DNA nucleotides.

Design of a gene-specific UPL assay is performed using ProbeFinder Software. User-defined target information is used to select the suitable Universal ProbelLibrary probe matched to a set of target-specific PCR primers. The UPL probe and the PCR primers together constitute the PCR assay for a given target gene.

ProbeFinder Software is available free at the web-based Assay Design Center at [www.universalprobelibrary.com](http://www.universalprobelibrary.com).

### How this Product Works

The Universal ProbelLibrary probes enable quantification of gene expression levels of a large number of organisms (human, primates, mouse, rat, *C. elegans*, *Drosophila*, Zebrafish, Rice, Maize, Yeast, *Arabidopsis*) and other organisms included in the NCBI Reference Sequence Database.

The Universal ProbelLibrary uses the hydrolysis probe format for real-time PCR detection. Hydrolysis probe assays are homogeneous 5' nuclease assays, with a single 3' non-extendable hydrolysis probe which is cleaved during PCR amplification. They are used to detect the accumulation of a specific target DNA sequence. Hydrolysis probes contain two labels in close proximity to each other: a fluorescent reporter dye at the 5' end and a quencher label at or near the 3' end. When the probe is intact, the fluorescent signal is suppressed by the quenching label. During PCR, when the probe is hybridized to its target sequence, the 5'→3' exonuclease activity of the Taq DNA polymerase cleaves the hydrolysis probe. Separating the reporter and the quencher "unquenches" the fluorescent reporter dye. During each PCR cycle, more of the released fluorescent dye accumulates, boosting the fluorescent signal.

## Assay Design Workflow

The selection of Universal ProbeLibrary probes and the design of primers for real-time PCR is made using the web-based ProbeFinder software available at the Universal ProbeLibrary Assay Design Center at [www.universalprobelibrary.com](http://www.universalprobelibrary.com).

*i* The ProbeFinder software has an extensive help function for use at any time during assay design.

- 1 Open your web browser and go to [www.universalprobelibrary.com](http://www.universalprobelibrary.com).
    - Select **Assay Design Center** from the navigation bar.
    - On the **Assay Design Center** start screen, select your organism of interest.

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  - 2 On the subsequent page, submit a sequence string or a sequence ID.
    - Choose whether you want to design an intron-spanning assay; the intron-spanning assay option is active by default. If you do not want to design intron-spanning assays, check the **Automatically select an intron-spanning assay** option for deselection.
    - When you want to design a multiplex assay with one of the UPL reference gene assays for human or mouse, check the option **Design multiplex PCR with reference gene**. Select one of the provided reference genes or select **Any**.
    - Click the **Design** button.

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  - 3 The software will select the best assay, which subsequently is presented on the Result Screen (together with the best suitable reference gene assay).

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  - 4 If for some reason the designed assay is not what you want, alternative and equally efficient assays can be found by clicking **More Assays**.

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- i* For a more detailed description of the ProbeFinder software, including advanced options such as assays that target specific exons or splice variants, see the *Universal ProbeLibrary Assay Design Guide*.



## 4.2. Quality Control

- All probes of the Universal ProbeLibrary have passed a real-time PCR performance test.
- All probes of the Universal ProbeLibrary are analyzed by anion-exchange HPLC and MALDI-MS to ensure purity and quality.

## 5. Supplementary Information

### 5.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
	<i>Information Note: Additional information about the current topic or procedure.</i>
	<b>Important Note: Information critical to the success of the current procedure or use of the product.</b>
① ② ③ etc.	Stages in a process that usually occur in the order listed.
① ② ③ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

### 5.2. Changes to previous version

Layout changes.  
Editorial changes.



## 5.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our homepage [lifescience.roche.com](http://lifescience.roche.com).

Product	Pack Size	Cat. No.
<b>Accessories general ( hardware )</b>		
LC Carousel Centrifuge 2.0	1 centrifuge plus rotor and rotor bucket (115 V)	03 709 507 001
	1 centrifuge plus rotor and rotor bucket (230 V)	03 709 582 001
<b>Consumables</b>		
LightCycler® Capillaries (20 µl)	5 x 96 capillaries, containing 5 boxes, each with 96 capillaries and stoppers, 5 boxes, each with 96 capillaries and stoppers	04 929 292 001
LightCycler® 480 Multiwell Plate 96, white	5 x 10 plates	04 729 692 001
LightCycler® 480 Multiwell Plate 384, white	5 x 10 plates	04 729 749 001
LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
<b>Instruments</b>		
LightCycler® 2.0 Instrument	1 instrument	03 531 414 001
LightCycler® 480 Instrument II	1 instrument	05 015 278 001
	1 instrument	05 015 243 001
<b>Reagents , kits</b>		
LightCycler® TaqMan® Master	1 kit, 96 reactions of 20 µl final volume each	04 535 286 001
	1 kit, 480 reactions of 20 µl final volume each	04 735 536 001
LightCycler® Uracil-DNA Glycosylase	50 µl, 100 U, (2 U/µl)	03 539 806 001
Universal ProbeLibrary Set, Human	1 set, 90 Universal ProbeLibrary probes, 125 µl, 10 µM, each	04 683 633 001
LightCycler® 480 Probes Master	5 x 1 ml, 2x conc., 5 x 100 reactions of 20 µl final volume each	04 707 494 001
	10 x 5 ml, 2x conc., 10 x 500 reactions of 20 µl final volume each	04 887 301 001
	1 x 50 ml, 2x conc., 5,000 reactions of 20 µl final volume each	04 902 343 001
Uracil-DNA Glycosylase, heat-labile	100 U, 1 U/µl	11 775 367 001
	500 U, 1 U/µl	11 775 375 001
Transcriptor First Strand cDNA Synthesis Kit	1 kit, 50 reactions, including 10 control reactions	04 379 012 001
	1 kit, 100 reactions	04 896 866 001
	1 kit, 200 reactions	04 897 030 001
Transcriptor Universal cDNA Master	1 kit, 100 reactions of 20µl final volume	05 893 151 001
FastStart TaqMan® Probe Master	2 x 1.25 ml, 100 reactions of 50 µl final volume each	04 673 409 001
	10 x 1.25 ml, 500 reactions of 50 µl final volume each	04 673 417 001
	10 x 5 ml, 2,000 reactions of 50 µl final volume each	04 673 433 001
FastStart Universal Probe Master (Rox)	2 x 1.25 ml, 100 reactions of 50 µl final volume each	04 913 949 001
	10 x 1.25 ml, 500 reactions of 50 µl final volume each	04 913 957 001
	10 x 5 ml, 2,000 reactions of 50 µl final volume each	04 914 058 001

## 5. Supplementary Information

### 5.4. Trademarks

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### 5.5. License Disclaimer

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### 5.6. Regulatory Disclaimer

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

### 5.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

### 5.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.  
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.

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