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Not for use in diagnostic procedures.



# Universal ProbeLibrary Reference Gene Assays

 **Version: 09**

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Reference gene-specific primers and probe for quantification of gene expression levels using dual-color real-time PCR

<b>Cat. No. 05 046 165 001</b>	Universal Probe Library Human ACTB Gene Assay
<b>Cat. No. 05 046 190 001</b>	Universal ProbeLibrary Mouse ACTB Gene Assay
<b>Cat. No. 05 046 211 001</b>	Universal ProbeLibrary Mouse GAPD Gene Assay
<b>Cat. No. 05 046 246 001</b>	Universal ProbeLibrary Human G6PD Gene Assay
<b>Cat. No. 05 190 541 001</b>	Universal ProbeLibrary Human GAPD Gene Assay

1 set for each assay for 200 reactions of 50 µl or  
500 reactions of 20 µl

**Store the set at –15 to –25°C.**

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# 1. General Information

## 1.1. Contents

Label	Function / Description	Catalog number	Content
“Universal Probelibrary Human ACTB Gene Assay, Primer” and “Universal Probelibrary Human ACTB Gene Assay, Probe”	Contains 4 vials per package: <ul style="list-style-type: none"> <li>Two vials, each containing a primer pair solution with a concentration of 20 µM for each primer.</li> <li>Two vials, each containing a 10 µM solution of the reference gene-specific probe.</li> </ul>	05 046 165 001	2 vials primer, 100 µl each 2 vials probe, 100 µl each
“Universal Probelibrary Human G6PD Gene Assay, Primer” and “Universal Probelibrary Human G6PD Gene Assay, Probe”	Contains 4 vials per package: <ul style="list-style-type: none"> <li>Two vials, each containing a primer pair solution with a concentration of 20 µM for each primer.</li> <li>Two vials, each containing a 10 µM solution of the reference gene-specific probe.</li> </ul>	05 046 246 001	2 vials, 100 µl each 2 vials, 100 µl each
“Universal Probelibrary Human GAPD Gene Assay, Primer” and “Universal Probelibrary Human GAPD Gene Assay, Probe”	Contains 4 vials per package: <ul style="list-style-type: none"> <li>Two vials, each containing a primer pair solution with a concentration of 20 µM for each primer.</li> <li>Two vials, each containing a 10 µM solution of the reference gene-specific probe.</li> </ul>	05 190 541 001	2 vials, 100 µl each 2 vials, 100 µl each
“Universal Probelibrary Mouse ACTB Gene Assay, Primer” and “Universal Probelibrary Mouse ACTB Gene Assay, Probe”	Contains 4 vials per package: <ul style="list-style-type: none"> <li>Two vials, each containing a primer pair solution with a concentration of 20 µM for each primer.</li> <li>Two vials, each containing a 10 µM solution of the reference gene-specific probe.</li> </ul>	05 046 190 001	2 vials, 100 µl each 2 vials, 100 µl each
“Universal Probelibrary Mouse GAPD Gene Assay, Primer” and “Universal Probelibrary Mouse GAPD Gene Assay, Probe”	Contains 4 vials per package: <ul style="list-style-type: none"> <li>Two vials, each containing a primer pair solution with a concentration of 20 µM for each primer.</li> <li>Two vials, each containing a 10 µM solution of the reference gene-specific probe.</li> </ul>	05 046 211 001	2 vials, 100 µl each 2 vials, 100 µl each

### 1.2. Storage and Stability

#### Storage Conditions (Product)

The set is shipped on dry ice.

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

Label	Storage
Universal ProbeLibrary Reference Gene Assay, Primer	Store at –15 to –25°C. For short-term storage for up to 1 month, the primers and probes may be stored at +2 to +8°C.
Universal ProbeLibrary Reference Gene Assay, Probe	<b>⚠ Avoid repeated freezing and thawing. After the first thaw, aliquot and store primers and probes.</b> <b>⚠ Keep Probe vials protected from light.</b>

### 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)

#### 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 165 probes of the Universal ProbeLibrary enable rapid and flexible quantification of virtually any transcript in the transcriptomes of any sequenced organism by real-time PCR assays on the LightCycler® 1.5, 2.0, and 480 Instruments or other commercially available real-time PCR instruments. Performance of the assay with the selected Universal ProbeLibrary probe follows established real-time PCR protocols.

Universal ProbeLibrary probes included in the reference gene assays are labeled with LightCycler® Yellow 555 at the 5' end and with a dark quencher dye near the 3' end, to enable dual-color assays together with the standard UPL probes which are labeled with FAM. Probes labeled with LightCycler® Yellow 555 can be detected using PCR instruments with excitation filters of 470 nm to 530 nm and emission filters of 550 nm to 640 nm. In a dual-color assay application with standard Universal ProbeLibrary assays, the following combination of filters is recommended: standard FAM or SYBR Green I filters and the next possible emission filter of a longer wavelength (usually 560, 568, or 640 nm) as the second channel.

Gene-specific expression quantification assays in combination with the UPL Reference Gene Assays for dual-color analysis are easily designed using the web-based ProbeFinder software at [universalprobelibrary.com](http://universalprobelibrary.com).

## 1.5. Preparation Time

### Typical run time

Using a LightCycler® 480 System or a LightCycler® Carousel-Based System with dedicated PCR reagents, the Universal ProbeLibrary Reference Gene Assays can be used with PCR protocols with run times of 50 to 70 minutes.

## 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, we recommend 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, we recommend 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. We recommend 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.

## Primers

Suitable concentrations of primers for your GOI (gene of interest) assay 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 primer 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 500 nM (final concentration). The recommended starting concentration is 200 nM. The 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 Universal ProbeLibrary Reference Gene Assay package contains enough primers and probe for 200 reactions in a 50 µl real-time PCR setup or 500 reactions in a 20 µl real-time PCR setup.

### Multiplex Experiment with the UPL Reference Gene Assays

The probes used for the UPL Reference Gene Assays contain the dye LightCycler<sup>®</sup> Yellow 555, whereas standard UPL probes contain a FAM dye. Most real-time PCR instruments that are able to read multiple dyes can distinguish these two dyes from each other. However, in most cases, the performance of a multiplex PCR with these two dyes requires that a calibration or color compensation is carried out with the real-time PCR instrument. Typically, this will require that the instruments are presented for these two dyes individually in order to optimize the algorithm used to separate the overlapping emission spectra for the dyes. For best results, consult the operator's manual for your instrument to obtain specific instructions on how to perform the color compensation or calibration.

### Dual-Color Assay

The Universal ProbeLibrary Reference Gene Assays enable relative quantification in multiplex PCR experiments. The ProbeFinder software allows design of multiplex PCR with your gene of interest (GOI) and one of the UPL reference gene assays for the organisms human, mouse, and rat. In order to design a multiplex PCR, the multiplex option must be selected in the target specification window on the Assay Design Center. The ProbeFinder software will subsequently design UPL assays for your GOI, while at the same time subjecting each of these designed assays to an *in silico* test to evaluate the ability of each assay to be multiplexed with one of the available UPL reference gene assays for the relevant organism. The output page will show the UPL assays and which UPL reference gene assays these may be multiplexed with. To perform the *in silico* multiplex PCR test, the software will take the following parameters into consideration:

- Primer-primer interactions – to avoid formation of primer-dimers
- Primer-probe interactions – to avoid probe-primer competition for binding sites or binding to each other
- Probe-probe interactions – to avoid probe-probe binding
- Probe-amplicon interactions – to avoid the “wrong” probe generating signal on the “wrong” amplicon
- *In silico* PCR with all 4 primers – to avoid amplification of undesired cDNA fragments

### Two-Assay Multiplexing

Designing a multiplex PCR experiment is a complex task. It is recommended to perform an *in vitro* validation of the designed multiplex PCR. The initial validation should include the following setup with 7 PCR reactions for each designed multiplex PCR:

PCR #	Reaction Type
1	GOI <sup>(1)</sup> – RG <sup>(2)</sup> multiplex
2	RG singleplex
3	GOI singleplex
4	RG primers with GOI probe
5	GOI primers with RG probe
6	NTC RG singleplex
7	NTC GOI singleplex

<sup>(1)</sup> Gene of Interest

<sup>(2)</sup> Reference Gene

Ideally, the assays should have the same Cp values when run in single- and multiplex. Furthermore, signal should not be generated when running a PCR using the UPL Reference Gene Primers with the GOI probe or a PCR using the GOI primers with the UPL Reference Gene Probe.

Finally, the designed multiplex assay may be validated by performing a template (cDNA) dilution series, that is, at least three individual PCRs with tenfold or twofold dilutions of the cDNA.

### Avoiding GOI Assay Inhibition from a Strong Reference Gene Assay

Occasionally, the GOI assay may show a slightly higher Cp value in multiplex than in singleplex. This may occur when an assay for a relatively high expressed reference gene (low Cp values) is multiplexed with an assay for a relatively low expressed GOI (high Cp values).

In these instances, it is recommended to try to inhibit the reference gene assay by using lower concentrations of the reference gene primer mix. Perform a titration of the reference gene primers using a dilution series and run it in multiplex with the GOI assay.

Analyze the resulting amplification curves for both the reference gene and the GOI assay. Identify the multiplex PCR experiment with the reference gene primer concentration where the GOI assay in multiplex performs similarly to the GOI assay in singleplex and the reference gene assay still maintains sufficient signal and good Cp values.

Choosing another of the GOI and/or reference gene assays suggested by ProbeFinder software could be a time-saving alternative to the titration experiment with the reference gene primers.



## 2.2. Protocols

### Protocols for Use with the LightCycler® 480 Instrument

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.

The following table shows the parameters that must be programmed for a LightCycler® 480 System PCR run using the LightCycler® 480 Probes Master and the LightCycler® 480 Multiwell Plate 96 or 384, respectively.

Setup					
Block Type		Reaction Volume [µl]			
96 (384)		20 (20)			
Detection Format	Excitation Filter	Emission Filter			
Dual Color Hydrolysis Probe / UPL Probe					
LightCycler® 480 Instrument I:					
FAM	483	533			
VIC / HEX / Yellow 555	523	568			
LightCycler® 480 Instrument II:					
FAM	465	510			
VIC / HEX / Yellow 555	533	580			
Program Name	Cycles	Analysis Mode			
Pre-Incubation	1	None			
Amplification	45	Quantification			
Cooling	1	None			
Temperature Targets					
	Target[°C]	AcquisitionMode	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)	–

**⚠ For data analysis, color compensation (CC) is required.**

- **LightCycler® 480 Instrument I: Requires an instrument-specific CC object generated by the user. For details, refer to the Operator's Guide for the LightCycler® 480 Instrument.**
- **LightCycler® 480 Instrument II: Use the universal CC object that is provided in the software.**

## 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. We recommend 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]
Primer Mix (UPL Reference Gene Assay)	20.0	0.4	400
Probe (UPL Reference Gene Assay)	10.0	0.4	200
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
LightCycler® 480 Probes Master, Water, PCR Grade (Vial 2)	–	3.0	–
<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.

## Protocols 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

**⚠ For data analysis, color compensation (CC) is required. For details see the LightCycler® Instrument Operator's Manual.**

**i** Channel settings for data analysis:

- Channel 530 and 560 when using the LightCycler® 2.0 Instrument.
- Channel 530 and 640 when using the LightCycler® 1.5 Instrument or lower version.

## 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).

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

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- 3 Pipette 10 µl from Vial 1a into Vial 1b.
  - i* Each Vial 1a contains enough enzyme for three vials of Reaction Mix.

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

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

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- 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. We recommend 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]
Primer Mix (UPL Reference Gene Assay)	20.0	0.4	400
Probe (UPL Reference Gene Assay)	10.0	0.4	200
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 TaqMan® Master, (Vial 1)	5x	4.0	1x
LightCycler® 480 TaqMan® Master, Water, PCR Grade (Vial 2)	–	9.0	–
<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 LightCycler® Capillary.
  - Add 5 µl of the cDNA template.

- 5 Seal each capillary with a stopper.

- 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.

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

## 2. How to Use this Product

### Protocols 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. The Universal ProbeLibrary Reference Gene Assays are compatible with real-time PCR instruments with excitation filters of 470 nm to 530 nm and emission filters of 550 to 640 nm.

**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.

### Preparation of the PCR Mix

Depending on the real-time PCR instrument you use, PCR reagents containing a reference dye (Rox) may be required. We recommend 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 for one 20 µl reaction mix by adding the following components in the order listed below:

Reagent	Conc. [µM]	Volume [µl]	Final conc. [nM]
Primer Mix (UPL Reference Gene Assay)	20.0	0.5	500
Probe (UPL Reference Gene Assay)	10.0	0.5	250
Primer (GOI) forward	20.0	0.5	500
Primer (GOI) reverse	20.0	0.5	500
UPL Probe (GOI)	10.0	0.5	250
FastStart Universal Probe Master (Rox) or FastStart TaqMan® Probe Master	2x	10.0	1x
Water, PCR Grade	–	2.5	–
<b>Total Volume</b>		<b>15.0</b>	

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

**4** 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, we recommend 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 <b>Automatically select an intron-spanning assay</b> , 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.

Universal ProbelLibrary assays for human and mouse targets can be combined with a Universal ProbelLibrary Reference Gene Assay for a dual-color assay. The multiplex option on the sequence input window allows you to design assays for your target gene(s) of interest, in combination with the available reference genes for the selected organism.

Universal ProbelLibrary probes included in the reference gene assays are 12-mer probes that are partially modified with LNA. They are labeled with LightCycler® Yellow 555 to enable dual-color assays together with the standard UPL probes, which are labeled with FAM.



## Assay Design Workflow

The selection of Universal ProbeLibrary probes and the design of primers for real-time PCR of gene transcripts 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 left side of the screen.
  - 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 for your gene of interest (GOI).
  - For dual-color assays, check the box **Design multiplex PCR with reference gene**. Select **Any** if you want ProbeFinder to find the most suitable reference gene assay for your gene of interest, or select one of the provided assays in the drop-down menu.
  - 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.
  - Check the **Design** button.

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- 3 ProbeFinder Software will design appropriate primers and select the best matching UPL probe for your target 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, see the *Universal ProbeLibrary Assay Design Guide*.



## 4.2. Quality Control

Primers and probes of Universal ProbeLibrary Reference Gene Assays are analyzed by anion-exchange HPLC and MALDI-MS to ensure purity and quality. Each Universal ProbeLibrary Reference Gene Assay has passed a real-time PCR performance test.

## 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

Editorial changes.

### 5.3. Ordering Information

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
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
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

FASTSTART, HIGH PURE, LIGHTCYCLER, MAGNA PURE and TAQMAN are trademarks of Roche.  
SYBR is a trademark of Thermo Fisher Scientific Inc..  
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### 5.5. License Disclaimer

For patent license limitations for individual products please refer to: [List of LifeScience products](#)

### 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

To ask questions, solve problems, suggest enhancements or report new applications, please visit our [Online Technical Support](#) Site.

To call, write, fax, or email us, visit [sigma-aldrich.com](http://sigma-aldrich.com) and select your home country. Country-specific contact information will be displayed.

