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Universal ProbeLibrary Probes 1 – 165

Version 13

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Short hydrolysis probes for quantification of gene expression levels by real-time PCR.

2 vials of Universal ProbeLibrary probe per package, 125 µl, 10 µM, each.

Store the probe vials at −15 to −25°C

⚠ Keep away from light!

Probe vials are shipped at ambient temperature

www.roche-applied-science.com

Table of Contents

PROTOCOL

PROTOCOL

1. What this Product Does

About this Product

The Universal ProbeLibrary is a powerful system for quantifying the expression level of virtually any transcript of a given organism using real-time qPCR assays. System components include 165 pretested, double-labeled real-time PCR detection probes and the web-based ProbeFinder Software. ProbeFinder Software is a fast and easy way to design gene-specific assays using gene-specific PCR primers and Universal ProbeLibrary (UPL) probes.

Universal ProbeLibrary (UPL) Probes are short hydrolysis probes, dual-labeled with a reporter fluorophore (FAM) and a dark 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. To maintain the hybridization stability and specificity required for qPCR probes, Locked Nucleic Acids (LNAs) are incorporated into the sequence of each UPL probe. For more information about UPL see Section 4.

Contents

Each Universal ProbeLibrary Probe package contains two vials. Each vial contains 125 µl of a 10 µM solution of a pretested probe.

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.

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 100 nM, each vial contains enough probe for 250 assays in a 50 µl or 625 assays in a 20 µl real-time PCR setup.

Storage and Stability

Store probe vials at −15 to −25°C until the expiration date printed on the label. For short-term storage (up to 1 month), the probes may be stored at +2 to +8°C.

Probe vials are shipped at ambient temperature.

Keep the probe vials away from light!

Avoid repeated freezing and thawing. After the first thawing, store the probes aliquoted.

Additional Equipment and Reagents Required

Additional reagents and equipment required to perform quantitative real-time PCR assays with the Universal ProbeLibrary probes include:

- Standard laboratory equipment
- For first strand cDNA synthesis:
  - Transcriptor First Strand cDNA Synthesis Kit*
- For use in combination with the LightCycler® Carousel-Based System:
  - LightCycler® 2.0 Instrument*, LightCycler® 1.5 Instrument, or lower version
  - LightCycler® TaqMan® Master*
  - LightCycler® Capillaries*
  - LC Carousel Centrifuge 2.0* (optional)
- For use in combination with the LightCycler® 480 System:
  - LightCycler® 480 Instrument 96 or 384*
  - LightCycler® 480 Probes Master*
  - LightCycler® 480 Multiwell Plate 96 or 384*
  - LightCycler® 480 Sealing Foil*
  - Standard swing-bucket centrifuge containing a rotor and adapters for multiwell plates
- 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)

Application

The Universal ProbeLibrary Probes are intended for life science research and enable 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, 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.

Gene specific expression quantification assays are easily designed using the web-based ProbeFinder software accessible at www.universalprobelibrary.com or via the Roche Applied Science home page, www.roche-applied-science.com.

Assay Time

A complete Universal ProbeLibrary assay, including assay design and real-time PCR reaction, needs approx. 2 days. The table below describes the basic workflow of a Universal ProbeLibrary assay:

Day 1
Decide on target sequence of interest, *e.g.*, entire transcript, exon, or other sequence.
Identify probes and primers using the ProbeFinder software.
Order primers from your preferred oligo supplier for overnight delivery. If you want to order a Universal ProbeLibrary probe based on the ProbeFinder result, you can directly switch to the Roche Applied Science Online Ordering site from the ProbeFinder software. Simply submit the probe name or the catalog number that can be found on the ProbeFinder result screen. In this case, extra delivery time for the probe has to be considered.
Day 2
Combine the relevant probe with the corresponding primers.
Perform real-time PCR.
Evaluate results.

2. How to Use this Product

2.1 Before You Begin

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. Pipet 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 N-Glycosylase.

Sample Material

Universal ProbeLibrary assays are designed for the amplification of cDNA.

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 use one of the following:

- MagNA Pure 96 Instrument with a dedicated MagNA Pure 96 reagent kit for high-throughput automated isolation
- MagNA Pure LC Instrument with a dedicated MagNA Pure LC reagent kit for medium-throughput automated isolation
- MagNA Pure Compact Instrument with a dedicated MagNA Pure Compact RNA isolation kit for low-throughput automated isolation
- High Pure RNA Isolation Kit for manual isolation

For further information, visit the Roche Applied Science home page at www.roche-applied-science.com.

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, Roche recommends 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.

Negative Control

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 a RT minus control. In the PCR reaction, use a RNA sample amount that is equivalent to the cDNA sample to be tested.

Primers

Use PCR primers at a final concentration of 200 – 900 nM. The recommended starting concentration is 400 nM each. The primer concentration may be optimized by varying the final concentration from 200 - 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.

The optimal primer concentration is the lowest concentration that results in the lowest Cp (or Ct) and an adequate fluorescence for a given target concentration.

Universal ProbeLibrary Probes

Use the probes at a final concentration of 100 - 200 nM. 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.
- The optimal probe concentration is the lowest concentration that results in the lowest Cp (or Ct) and an adequate fluorescence for a given target concentration.

Passive Reference Dye

The LightCycler® 480 Instrument and the LightCycler® Carousel-Based 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. Refer to the Operator's Manual of your real-time PCR instrument to obtain this information.

Preparation of the PCR Mix

Use the LightCycler® 480 Probes Master for performing UPL assays on LightCycler® 480 Instruments.

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

Do not touch the surface on the LightCycler® 480 Multiwell Plate when handling it.

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 suitable 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:

Component	Conc.	Vol.	Final Conc.
H_2O, PCR grade (Vial 2)	-	3.8 µl	-
Primer (GOI) forward	20 µM	0.4 µl	400 nM
Primer (GOI) reverse	20 µM	0.4 µl	400 nM
UPL probe (GOI)	10 µM	0.4 µl	200 nM
LightCycler® 480 Probes Master (Vial 1)	2 × conc.	10.0 µl	1 × conc.
Total Volume		**15.0 µl**	

3. Mix carefully by pipetting up and down. Do not vortex.
4. • Pipet 15 µl of the PCR mix into each well of the LightCycler® 480 Multiwell Plate.
   • Pipet 5 µl of template (cDNA) to the PCR mix of each well.
5. Seal the Multiwell Plate with a LightCycler® 480 Sealing Foil.
6. Centrifuge the Multiwell Plate for 2 min at 1,500 × *g* in a standard swing-bucket centrifuge, using a rotor for multiwell plates and suitable adaptors. Make sure to balance it 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 below.

LightCycler® 480 Instrument Protocol for the 96 Multiwell Plate Format

For more details on how to program the experimental protocol, see the Operator's Guide of the LightCycler® 480 Instrument.

The following table shows a typical protocol for using the LightCycler® 480 System with Multiwell Plates 96 and a 20 µl reaction volume with the **LightCycler® 480 Probes Master**. It should contain the following programs:

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

Setup			
Detection Format		**Block Type**	**Reaction Volume**
Monocolor Hydrolysis Probe / UPL Probe		96	20 µl
Filter combination: dynamic Mode FAM 483 – 533 or 465 – 510 respectively, for LightCycler® 480 Instrument Version I or II			
Programs			
Program Names		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)
Pre-Incubation			
95	None	00:10:00	4.4
Amplification			
95	None	00:00:10	4.4
60	None	00:00:30	2.2
72	Single	00:00:01	4.4
Cooling			
40	None	00:00:30	2.2

LightCycler® 480 Instrument Protocol for the 384 Multiwell Plate Format

For more details on how to program the experimental protocol, see the Operator's Guide of the LightCycler® 480 Instrument.

The following table shows a typical PCR protocol for using the LightCycler® 480 System with Multiwell Plates 384 and a 10 µl reaction volume with the **LightCycler® 480 Probes Master**. It should contain the following programs:

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

Setup			
Detection Format		**Block Type**	**Reaction Volume**
Monocolor Hydrolysis Probe / UPL Probe		348	10 µl
Filter combination: dynamic Mode FAM 483 – 533 or 465 – 510 respectively, for LightCycler® 480 Instrument Version I or II			
Programs			
Program Names		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)
Pre-Incubation			
95	None	00:10:00	4.8
Amplification			
95	None	00:00:10	4.8
60	None	00:00:30	2.5
72	Single	00:00:01	4.8
Cooling			
40	None	00:00:30	2.5

Preparation of the Master Mix

Use the LightCycler® TaqMan® Master for performing UPL assays on LightCycler® Carousel-Based Instruments. Prepare the 5× Master Mix of the **LightCycler® TaqMan® Master** as described below:

1. Thaw one vial of "Reaction Mix" (Vial 1b, red cap).
2. Briefly centrifuge one Vial 1a ("Enzyme", white cap) and one thawed Vial 1b ("Reaction Mix", red cap, from Step1).
3. Pipet 10 µl from Vial 1a (white cap) into Vial 1b (red cap).
   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 (red cap) with the new label (Vial 1: Master Mix) provided with the kit.

The volume of the resulting Master Mix (5× 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 suitable 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:

Component	Conc.	Volume	Final conc.
H_2O, PCR grade (Vial 2, colorless cap)	-	9.8 µl	-
Primer (GOI) forward	20 µM	0.4 µl	400 nM
Primer (GOI) reverse	20 µM	0.4 µl	400 nM
UPL probe (GOI)	10 µM	0.4 µl	200 nM
Master Mix, (Vial 1, red cap)	5 × conc.	4.0 µl	1 × conc.
Total volume		**15.0 µl**	

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

❹	• Pipet 15 µl of the PCR mix into each LightCycler® Capillary. • Add 5 µl of the cDNA template.
❺	Seal each capillary with a stopper.
❻	• Centrifuge the LightCycler® Sample Carousel containing the capillaries in the LC Carousel Centrifuge. • Alternatively, place the adapters containing the capillaries in a standard benchtop microcentrifuge. Centrifuge at 700 × *g* for 5 s (3.000 rpm in a standard benchtop microcentrifuge). Transfer the capillaries to the sample carousel of the LightCycler® Instrument.
❼	Start the LightCycler® Instrument run by using the PCR program as described below.

Instrument Protocol for LightCycler® Carousel-Based Systems

For more details on how to program the experimental protocol, see the Operator's Guide of your LightCycler® Instrument.

The following table shows a typical PCR protocol that uses the **LightCycler® TaqMan® Master.** It should contain the following programs:

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

Programs				
Program Name			Cycles	Analysis Mode
Pre-Incubation			1	None
Amplification			45	Quantification
Cooling			1	None
Temperatur Targets				
Target (°C)	Hold (hh:mm:ss)	Ramp Rate (°C/s)	Acquisition Mode	
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	

General Remarks

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 for each respective master mix.

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.

❶ • 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.

❷ • Prepare the PCR mix in a suitable 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:

Component	Conc.	Volume	Final Conc.
H_2O, PCR grade (Vial 2)	-	3.8 µl	-
Primer (GOI) forward	20 µM	0.4 µl	400 nM
Primer (GOI) reverse	20 µM	0.4 µl	400 nM
UPL probe (GOI)	10 µM	0.4 µl	200 nM
FastStart Universal Probe Master (Rox) or FastStart TaqMan® Probe Master	2 × conc.	10.0 µl	1 × conc.
Total volume		**15.0 µl**	

❸ Mix carefully by pipetting up and down. Do not vortex.

❹ • Pipet 15 µl of the PCR mix into each reaction device of your real-time PCR system.
• Pipet 5 µl of template (cDNA) to the PCR mix in each reaction device.

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

Instrument Protocol

Use a standard real-time PCR protocol for hydrolysis probes. 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).

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

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.

The ProbeFinder software has an extensive help function that may be visited whenever in doubt.

1. • Open your web browser and go to 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.

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. When you do not want to design intron-spanning assays, tick 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, mouse or rat, tick the option ''Design multiplex PCR with reference gene''. Select one of the provided reference genes or select ''Any''.
   • Click the “Design” button.

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

4. • If for some reason the designed assay is different from what you desire, alternative and often just as efficient assays are available upon clicking the “More Assays”.

For a more detailed description of the ProbeFinder software, including advanced options, such as assays that target specific exons or splice variants, visit www.universalprobelibrary.com for the Universal ProbeLibrary Assay Design Guide.

For further troubleshooting hints, visit our Online Technical Support Site at www.technical-support.roche.com..

	Possible Cause	Recommendation
No positive fluorescent signal during real-time PCR	Insufficient template amount	• Add more cDNA when working with very low abundance transcripts, or • 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. For this reason, Roche recommends diluting the cDNA at least 1:5 fold for a single PCR reaction.
	Wrong detection channels or parameters	Check your PCR protocol.
Log-linear phase of amplification just begins at the end of the cycling program	Number of cycles in the PCR protocol is too low	Run a PCR protocol with 45 or even 50 cycles.
	Insufficient template amount	• Add more cDNA when working with very low abundance transcripts, or • Include more RNA in your reverse transcription reaction.
RT minus control with positive signal	Amplification of remaining genomic DNA in the RNA sample preparation	• Improve your RNA preparation e.g., 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 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

How this Product Works

The Universal ProbeLibrary 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 ProbeLibrary uses the hydrolysis probe format for real-time PCR detection. Hydrolysis probe assays are homogenous 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 ProbeLibrary Principle

The Universal ProbeLibrary 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 ProbeLibrary (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 ProbeLibrary 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.

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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

Text Conventions To make information consistent and easier to read, the following text conventions are used in this document:

Text Convention	Usage
Numbered instructions labeled ❶, ❷ etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Applied Science.

Symbols In this document, the following symbols are used to highlight important information:

Symbol	Description
🔍	Information Note: Additional information about the current topic or procedure.
⚠	Important Note: Information critical to the success of the procedure or use of the product.

Abbreviations In this document, the following abbreviations are used:

Abbreviation	Meaning
Cp	Crossing point
Ct	Threshold cycle
GOI	Gene of Interest
LNA	Locked Nucleic Acid
MWP	Multiwell plate
NA	Nucleic acid (RNA or DNA)
PCR	Polymerase Chain Reaction
qPCR	quantitative real-time PCR
RG	Reference Gene
RT-PCR	Reverse transcription polymerase chain reaction
UNG	Uracil-DNA Glycosylase
UPL	Universal ProbeLibrary

5.2 Changes to Previous Version

- Probe vials are shipped at ambient temperature.

5.3 Ordering Information

	Product	Pack Size	Cat. No.
Reagents for cDNA Synthesis	Transcriptor First Strand cDNA Synthesis Kit	1 kit for up to 50 reactions, including 10 control reactions	04 379 012 001
		1 kit for up to 100 reactions	04 896 866 001
		1 kit for up to 200 reactions	04 897 030 001
	Transcriptor Universal cDNA Master	100 reactions	05 893 151 001
Universal ProbeLibrary Sets and Single Probes	Universal ProbeLibrary Set, Human	1 set of 90 Universal ProbeLibrary probes, 125 µl, 10 µM, each	04 683 633 001
	Universal ProbeLibrary Set, Mouse	1 set of 90 Universal ProbeLibrary probes, 125 µl, 10 µM, each	04 683 641 001
	Universal ProbeLibrary Set, Rat	1 set of 90 Universal ProbeLibrary probes, 125 µl, 10 µM, each	04 683 650 001
	Universal ProbeLibrary Extension Set, Probes #91 - #165	1 set of 75 Universal ProbeLibrary probes, 125 µl, 10 µM, each	04 869 877 001
	Universal ProbeLibrary Probes	2 vials of Universal ProbeLibrary probe per package, 125 µl, 10 µM, each	For details, visit www.universal-probelibrary.com

	Product	Pack Size	Cat. No.
Universal ProbeLibrary Reference Gene Assays	Universal ProbeLibrary Set, Human Reference Gene Assays	1 set 100 reactions of 50 µl or 250 reactions of 20 µl for each reference gene	05 046 114 001
	Universal ProbeLibrary Human PBGD Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 046 149 001
	Universal ProbeLibrary Human G6PD Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 046 246 001
	Universal ProbeLibrary Human ACTB Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 046 165 001
	Universal ProbeLibrary Human PGK1 Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 046 173 001
	Universal ProbeLibrary Human TBP Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 189 284 001
	Universal ProbeLibrary Human β2M Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 189 390 001
	Universal ProbeLibrary Human PPIA Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 189 268 001
	Universal ProbeLibrary Human HPRT Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 046 157 001
	Universal ProbeLibrary Human GUSB Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 190 525 001
	Universal ProbeLibrary Human GAPD Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 190 541 001
	Universal ProbeLibrary Mouse ACTB Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 046 190 001
	Universal ProbeLibrary Mouse GAPD Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 046 211 001
	Universal ProbeLibrary Rat ACTB Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 046 203 001
	Universal ProbeLibrary Rat GAPD Gene Assay	1 set 200 reactions of 50 µl or 500 reactions of 20 µl	05 046 220 001

	Product	Pack Size	Cat. No.
LightCycler® 480 Instruments and Accessories	LightCycler® 480 Instrument II	1 instrument (96-well)	05 015 278 001
		1 instrument (384-well)	05 015 243 001
	LightCycler® 480 Multiwell Plate 96, white	5 ×10 plates (includes sealing foils)	04 729 692 001
	LightCycler® 480 Multiwell Plate 384, white	5 ×10 plates (includes sealing foils)	04 729 749 001
	LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
LightCycler® 480 Kit for PCR	LightCycler® 480 Probes Master	5 × 1 ml (5 × 100 reactions, 20 µl each)	04 707 494 001
		10 × 5 ml (10 × 500 reactions, 20 µl each)	04 887 301 001
		1 × 50 ml (5,000 reactions, 20 µl each)	04 902 343 001
LightCycler® Carousel-Based Instrument and Accessories	LightCycler® 2.0 Instrument	1 instrument plus related products and data station	03 531 414 001
	LightCycler® Capillaries (20 µl)	1 pack containing 5 boxes, each with 96 capillaries and stoppers	04 929 292 001
	LC Carousel Centrifuge 2.0	1 centrifuge plus rotor and rotor bucket (230 V)	03 709 582 001
		1 centrifuge plus rotor and rotor bucket (115 V)	03 709 507 001
	LightCycler® Software 4.1	1 software package	04 898 915 001
LightCycler® Carousel-Based System Kit for PCR	LightCycler® TaqMan® Master	1 kit for 96 reactions of 20 µl final reaction volume	04 535 286 001
		1 kit for 480 reactions of 20 µl final reaction volume	04 735 536 001
Kits for PCR with other real-time PCR instruments	FastStart Universal Probe Master (Rox)	2 × 1.25 ml for 250 reactions of 20 µl final reaction volume	04 913 949 001
		10 × 1.25 ml for 1,250 reactions of 20 µl final reaction volume	04 913 957 001
		10 × 5 ml for 5,000 reactions of 20 µl final reaction volume	04 914 058 001
	FastStart TaqMan® Probe Master	2 × 1.25 ml for 100 reactions of 50 µl final reaction volume	04 673 409 001
		10 × 1.25 ml for 500 reactions of 50 µl final reaction volume	04 673 417 001
		10 × 5 ml for 2,000 reactions of 50 µl final reaction volume	04 673 433 001
Reagents for Prevention of Carryover Contamination	LightCycler® Uracil-DNA Glycosylase	50 µl 100 U (2 U/µl)	03 539 806 001
	Uracil-DNA Glycosylase, heat-labile	100 U	11 775 367 001
		500 U	11 775 375 001
RealTime ready Assays and Panels	RealTime ready Catalog and Designer Assays	Function tested RT-qPCR Assays for human, mouse and rat genes of your choice (300 reactions)	For details, visit www.realtime-ready.roche.com
	RealTime ready Custom Panel 96 or 384	LightCycler® 480 Multiwell Plates 96 or 384 containing RealTime ready Assays of your choice	For details, visit www.realtime-ready.roche.com

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