

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



RealTime ready Cell Lysis Kit

 **Version: 05**
Content version: July 2017

Easy-to-use reagent for lysing cells, prior to reverse transcription.

Cat. No. 05 943 523 001	1 kit
	500 reactions of 40 µl final volume each
	500 lysis reactions with a final reaction volume of 40 µl each
Cat. No. 06 366 821 001	1 kit
	50 reactions of 40 µl final volume each
	50 lysis reactions with a final reaction volume of 40 µl each

Store the kit at –15°C to –25°C until the expiration date printed on the label.

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1. General Information

1.1. Contents

Number of Tests

The kit is designed for:

- Cat. No. 05 943 523 001: 500 lysis reactions, with a final volume of 40 µl each
- Cat. No. 06 366 821 001: 50 lysis reactions, with a final volume of 40 µl each

Kit Contents

Vial/Bottle	Cap	Label	Function / Description	Catalog Number	Content
1	colorless	RealTime ready Lysis Buffer	▪ Easy-to-use Lysis Buffer	05 943 523 001	1 bottle, 20 ml
				06 366 821 001	1 bottle, 2 ml
2	colorless	Protector RNase Inhibitor	▪ Contains Protector RNase Inhibitor (40 U/µl) ▪ To prevent RNA degradation during lysis of the cells	05 943 523 001	1 vial, 250 µl
				06 366 821 001	1 vial, 25 µl
3	colorless	Thermolabile Nuclease	▪ To degrade double-stranded DNA during the Reverse Transcription reaction (optional)	05 943 523 001	1 vial, 250 µl
				06 366 821 001	1 vial, 25 µl

1.2. Storage and Stability

Storage Conditions (Product)

 **The kit is shipped on dry ice**

 **After first thawing, the RealTime ready Lysis Buffer (Bottle 1) can be stored at +2 to +8°C for at least 2 months.**

Storage Conditions (Working Solution)

Once the kit is opened, store the kit components as described in the following table:

Vial/Bottle	Cap	Label	Storage
1	colorless	RealTime ready Lysis Buffer	Store at –15°C to –25°C  After first thawing, the RealTime ready Lysis Buffer (Bottle 1) can be stored at +2 to +8°C for at least 2 months.
2	colorless	Protector RNase Inhibitor	Store at –15°C to –25°C
3	colorless	Thermolabile Nuclease	Store at –15°C to –25°C

1.3. Additional Equipment and Reagents Required

- Standard laboratory equipment
- Pipettes and nuclease free, aerosol-preventative tips
- Sterile reaction tubes (Eppendorf) for preparing the Cell Lysis Reagent, master mixes and dilutions
- Phosphate Buffered Saline (PBS) for washing the cells

For Reverse Transcription:

- Transcriptor Reverse Transcriptase*
- Transcriptor First Strand cDNA Synthesis Kit*
- Transcriptor High Fidelity cDNA Synthesis Kit*
- Transcriptor Universal cDNA Master*
- Primer for cDNA Synthesis* or Primer “random”*

For Two-Step RT-PCR:

- LightCycler® 480 Probes Master*
- RealTime ready DNA Probes Master*

For One-Step RT-PCR:

- LightCycler® 480 RNA Master Hydrolysis Probes*

For Gene Expression:

- Universal ProbeLibrary assays[§]
- RealTime ready Focus Panels[§]
- RealTime ready Custom Single Assays[§]
- RealTime ready Custom Panels[§]

* available from Roche Applied Science; see Ordering Information for details.

§ for detailed information, please visit www.universalprobelibrary.com and www.realtimeready.roche.com.

1.4. Application

RealTime ready Cell Lysis Kit is designed for the lysis of cultured cells. Cell lysates can then be directly used for cDNA synthesis, using a reverse transcription kit for cDNA synthesis*, followed by an appropriate PCR reagent for use on the LightCycler® Instruments or standard thermal block cyclers. The quality of RNA isolated is suitable for relative quantification of mRNA by RT-PCR, especially on the LightCycler® Instruments.

Cell lysates can be processed immediately for RT-PCR, or stored frozen at –15 to –25°C, or stored at +2 to +8°C for a maximum of 2 days.

1.5. Preparation Time

Procedure	Time(min)
Prepare Cell Lysis Reagent	5
Wash cells with cold PBS	5
Add Cell Lysis Reagent and incubate	5
Total Time for Cell Lysis	15
Reverse Transcription Setup	15
Reverse Transcription	45
PCR Setup	15
Real-Time PCR with the Universal ProbeLibrary	45
Total Time from Lysis to Real-Time PCR Results	135

2. How to Use this Product

2.1. Before you Begin

Sample Materials

To obtain optimal results in downstream procedures, especially in real-time RT-PCR assays on the LightCycler® Instruments, do not process samples larger than this kit is designed to handle.

The optimal amount of sample material is as follows:

- Seed an appropriate number of adherent cells and grow them overnight.
- The maximal cell number and the volume of Cell Lysis Reagent depends on the type of culture plate used.

For a 96-well plate:

3 to 3×10^4 cells per well are recommended. Depending on the cell size, up to 10^5 cells can be used. Although, higher concentrations may result in loss of signal, as the maximal number of adherent cells is restricted by cell density.

Cell Types that we have tested:

Table 1 lists the adherent cell types that we have tested with the RealTime ready Cell Lysis Kit

Cell Line	Organism	Disease/ Tissue
BT-549	human	breast ductal carcinoma
HeLa	human	cervical adenocarcinoma
HepG2	human	hepatocellular carcinoma
HT-29	human	colorectal adenocarcinoma
HT-1080	human	connective tissue fibrosarcoma
HUV-EC-C	human	vascular endothelium from umbilical vein
MCF7	human	breast adenocarcinoma
MDA-MB-231	human	breast adenocarcinoma
MDA-MB-468	human	breast adenocarcinoma
NIH/3T3	human	embryonic fibroblast
SK-BR-3	human	breast adenocarcinoma

Control Reactions

Negative Control

Always run a negative extraction control with the samples. To prepare a negative extraction control, replace the cells with PCR-grade water*, or PBS*. In case of a contamination problem, this can be observed with the negative control. Also, run a negative (no template) control and a RT negative (no RT) control in the RT-PCR. To prepare a negative (no template) control, replace the cell lysate with PCR-grade water*. To prepare a RT negative (no RT) control, perform PCR of the RNA template, in combination with e.g. LightCycler® 480 Probes Master*, or RealTime ready DNA Probes Master*. In this experimental setup, the reverse transcription step is omitted, thus any PCR product generated is a signal for DNA contamination of the RNA template preparation.

Safety Information

I) Handling Requirements

- Complete each phase of the RT-PCR workflow before proceeding to the next phase. For example, you should finish RT-PCR sample preparation before starting RT-PCR set-up. Sample preparation, RT-PCR set-up and the RT-PCR run itself should also be performed in separate locations.
- Do not pool reagents from different lots or from different bottles of the same lot.
- Do not use a kit after its expiration date has passed.
- Cell Lysis Buffer (bottle 1) contains guanidinium salts, which are irritants. Do not let Cell Lysis Buffer touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If you spill the reagent, dilute the spill with water before wiping it up.
- Do not allow the Cell Lysis Buffer to mix with sodium hypochlorite (bleach) solution or strong acids. This mixture can produce a highly toxic gas.

II) Laboratory Procedures

- Use RNase-free Techniques: RNase contaminated reagents and reaction vessels will degrade template RNA.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents. Change disposable gloves frequently.
- Do not contaminate the reagents with bacteria, virus or ribonucleases. Use disposable pipettes and RNase-free pipette tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Avoid touching surfaces or materials that could cause RNase carryover.
- Use only the reagents provided in this kit. Substitutions may introduce RNases.
- Clean and decontaminate work areas and instruments, including pipettes, with commercially available decontamination reagents.
- Use only new RNase-free aerosol-blocking pipette tips and microcentrifuge tubes.
- Use a work area specifically designated for RNA work and if possible use reaction vessels and pipettes dedicated only for work with template RNA.
- Regarding precautions for safe handling of RNA, see the Roche Life Science Lab FAQs (<https://lifescience.roche.com/labfaqs>).
- Wash hands thoroughly, after handling samples and reagents.

III) Waste Handling

- Discard unused reagents and waste in accordance with country, federal, state and local regulations.
- Material Safety Data Sheets (MSDS) are available on the Roche Life Science homepage (<https://lifescience.roche.com>), or upon request from the local Roche office.

Working Solution

Preparation of the Cell Lysis Reagent

Follow this procedure to prepare Cell Lysis Reagent for one well:

- ❶ Thaw the frozen Lysis Buffer (bottle 1) at 37°C, until the solution is clear, then equilibrate on ice.
- ❷ To ensure maximal recovery of all the contents, briefly spin the Protector RNase Inhibitor (vial 2) in a microcentrifuge before opening and mix carefully by pipetting up and down.
- ❸ In a 1.5 ml reaction tube on ice, prepare the Cell Lysis Reagent per well, by adding the following components in the order mentioned below:

Component	Volume for one well [μl]	Final conc.
RealTime ready Lysis Buffer (bottle 1)	39.5	1x
Protector RNase Inhibitor (vial 2)	0.5	20 U
Total volume	40.0	

⚠ **To prepare the Cell Lysis Reagent for more than one well, multiply the amount in the “Volume” column above by z , where z = the number of wells to be lysed + 5% additional wells.**

2.2. Protocols

Harvesting and Lysing Cells

Harvesting and Lysing Adherent Cells in a 96-well Plate.

Follow the procedure below to harvest and lyse adherent cells:

- 1 Carefully aspirate culture medium from the wells and wash each well with 100 µl ice-cold PBS.
i It is recommended to check that the cells are still attached to the culture plate.

- 2 Aspirate PBS from the wells and add into each well, 40 µl Cell Lysis Reagent (see section **Preparation of the Cell Lysis Reagent**).

- 3 Proceed to RT-PCR setup, or seal the plate with an adhesive foil and store the cell lysates for later analysis.
⚠ The lysates can be stored at +2 to +8°C for a maximum of 2 days, or at –15 to –25°C for several months, prior to RT-PCR, without any degradation of RNA.
i As the Protector RNase Inhibitor loses activity during storage at –15 to –25°C, it is extremely important to proceed with cDNA synthesis immediately after thawing.

Harvesting and Lysing Suspended/Pelleted Cells

Follow the procedure below to harvest and lyse suspended/pelleted cells:

- 1 Detach the cells, if necessary and pipette up to 10⁵ cells into microcentrifuge tubes.

- 2 Pellet the cells at +4°C, aspirate the culture medium and wash the cells with 100 µl ice-cold PBS.

- 3 Re-pellet the cells, aspirate PBS and add into each microcentrifuge tube, 40 µl Cell Lysis Reagent (see section **Preparation of the Cell Lysis Reagent**).

- 4 Incubate the microcentrifuge tubes for 5 min at room temperature.

- 5 Proceed to RT-PCR setup, or seal the plate with an adhesive foil and store the cell lysates for later analysis.
⚠ The lysates can be stored at +2 to +8°C for a maximum of 2 days, or at –15 to –25°C for several months, prior to RT-PCR, without any degradation of RNA.
i As the Protector RNase Inhibitor loses activity during storage at –15 to –25°C, it is extremely important to proceed with cDNA synthesis immediately after thawing.

Lysing Adherent Cells in a 384-well Plate

Use 10 µl of the Cell Lysis Reagent (from section **Preparation of the Cell Lysis Reagent**), instead of 40 µl.

cDNA Synthesis

The following procedure describes synthesis of first-strand cDNA for a two-step RT-PCR, using the provided Thermolabile DNase (vial 3) and the Transcriptor First Strand cDNA Synthesis Kit (Cat. No. 04 896 866 001).

i Including the Thermolabile DNase (vial 3) in the reverse transcription reaction is optional, especially if the PCR primers are designed to exclude the amplification of genomic DNA.

⚠ Program the thermal cycler with the reverse transcription program before preparing the reaction mixes.

To minimize the risk of RNase contamination, autoclave all vessels and pipette tips that will be used in the cDNA synthesis reaction. Wear gloves at all times.

1 Thaw all necessary components and place them on ice. To ensure recovery of all the contents, briefly spin all vials in a microcentrifuge before opening and mix carefully by pipetting up and down.

i Keep all reagents on ice after thawing.

2 In a 1.5 ml reaction tube on ice, prepare the RT mix per 20 µl reaction, by adding the following components in the order mentioned below:

Component	1 reaction [µl]	100 reactions[µl]	Final conc.
H ₂ O, PCR grade (vial 7, colorless cap)	8.5	850	–
Random Hexamer Primer (vial 6, blue cap)	2.0	200	60 µM
Transcriptor RT Reaction Buffer (5×; vial 2, colorless cap)	4.0	400	1x
Protector RNase Inhibitor (vial 3, colorless cap)	0.5	50	20 U
Deoxynucleotide Mix (vial 4, yellow/purple cap)	2.0	200	1 mM
Thermolabile DNase ⁽¹⁾ (vial 3, colorless cap)	0.5	50	1 U
Transcriptor Reverse Transcriptase (vial 1, red cap)	0.5	50	10 U
Total volume	18	1,800 µl	

i For setting up single reverse transcription reactions, refer to the package insert of the Transcriptor First Strand cDNA Synthesis Kit.

⁽¹⁾ Optional: if the Thermolabile DNase is not included, the volume of H₂O, PCR grade must be increased.

3 Mix the RT mix well, by vortexing. Spin the tube briefly in a microcentrifuge.

4 Pipette 18 µl RT mix into each well of a PCR plate/reaction tube.

5 Add 2 µl of the cell lysate.

6 Seal the PCR plate, or cap the reaction tubes.

7 Incubate the reaction for 10 min at +29°C, to enable the Thermolabile DNase to incubate and degrade the double-stranded DNA.

8 Incubate the reaction for at least 15 min at +55°C (maximum 45 min), to enable the Transcriptor Reverse Transcriptase to generate cDNA.

9 Incubate the reaction for 5 min at +85°C, to inactivate the Transcriptor Reverse Transcriptase. Place the plate/tube on ice.

10 At this point, the plate/tube may be stored at +2 to +8°C for 1 to 2 h, or at –15 to –25°C for longer storage. Alternatively, the cDNA can be processed for PCR, immediately.

Real-Time PCR using the LightCycler® System

The following procedure is an example for using with the LightCycler® 480 Probes Master (Cat. Nos. 04 707 494 001, 04 887 301 001, or 04 902 343 001) with the Universal ProbeLibrary. If using one of our other LightCycler® Master mixes, please refer to the respective package insert for details on setting up the instrument and PCR run.

i For detailed information regarding designing qPCR assays using the Universal ProbeLibrary or RealTime ready qPCR Assays, please visit www.universalprobelibrary.com and www.realtimeready.roche.com.

! **Program the instrument before preparing the reaction mixes.**

Protocol and PCR Setup for use with the LightCycler® 480 Probes Master

The following table shows the PCR parameters that must be programmed for a LightCycler® 480 System PCR run with the LightCycler® 480 Probes Master using a LightCycler® 480 Multiwell Plate 96 and the Universal ProbeLibrary.

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

Setup				
Block Type		Reaction Volume [μl]		
96		20		
Programs				
Program Name		Cycles	Analysis Mode	
Initial Denaturation		1	None	
Amplification		45	Quantification	
Cooling		1	None	
Temperature Targets				
	Target[°C]	AcquisitionMode	Hold[hh:mm:ss]	Ramp Rate[°C/s]
Initial Denaturation	95	None	00:10:00	4.4
Amplification	95	None	00:00:10	4.4
	60	Single	00:00:30	2.2
	72	None	00:00:01	4.4
Cooling	40	None	00:00:30	2.2

Preparation of the PCR Mix

Follow the procedure below to prepare one 20 µl standard reaction.

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

1 Thaw the solutions of the LightCycler® 480 Probes Master and to ensure recovery of all the contents, briefly spin vials in a microcentrifuge before opening.

2 Mix carefully by pipetting up and down and store on ice.

3 In a 1.5 ml reaction tube on ice, prepare the PCR mix for one 20 µl reaction by adding the following components in the order listed below:

Component	Conc.	Volume[µl]	Final Conc.
H ₂ O, PCR Grade (vial 2, colorless cap)	—	3.8	—
Forward Primer	20 µM	0.4	400 nM
Reverse Primer	20 µM	0.4	400 nM
Universal ProbeLibrary Probe	10 µM	0.4	200 nM
LightCycler® 480 Probes Master, 2× conc. (vial 1, red cap)	2×	10.0	1×
Total volume		1.0	

⚠ To prepare the PCR Mix for more than one reaction, multiply the amount in the “Volume” column above by z, where z = the number of reactions to be run + two additional reactions.

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

5 Pipette 15 µl PCR mix into each well of the LightCycler® 480 Multiwell Plate.

6 Add 5 µl of the cDNA template.

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

8 Place the Multiwell Plate in a centrifuge and balance it with a suitable counterweight (e.g., another Multiwell Plate).

9 Centrifuge for 2 min at 1,500 × g in a standard swing-bucket centrifuge, containing a rotor for multiwell plates with suitable adapters.

10 Load the Multiwell Plate into the LightCycler® 480 Instrument.

11 Start the PCR program described above.

3. Results

The following results were obtained using the RealTime ready Cell Lysis Kit with HT-29 and MCF7 cells, followed by reverse transcription using the Transcriptor cDNA Synthesis Kit. Amplification and detection was performed on the LightCycler® 480 Instrument with primers and Universal ProbeLibrary probes, specific for glucose-6-phosphate-dehydrogenase (G6PDH) and beta-2-microglobulin (b2M) and LightCycler® 480 Probes Master, using a LightCycler® 480 Multiwell Plate 96.

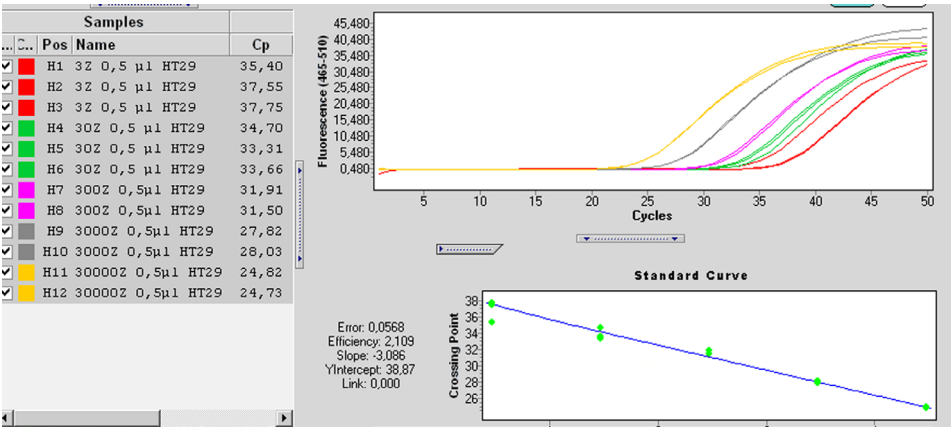


Fig. 1: RT-PCR analysis using the LightCycler® 480 Instrument of HT-29 cell lysate from 3 (far right) to 30,000 (far left) cells per well, targeting G6PDH. cDNA was synthesized from the HT-29 cell lysates using the Transcriptor cDNA Synthesis Kit. Detection of the PCR product was performed with Universal ProbeLibrary Probe #82 and primers specific for G6PDH.

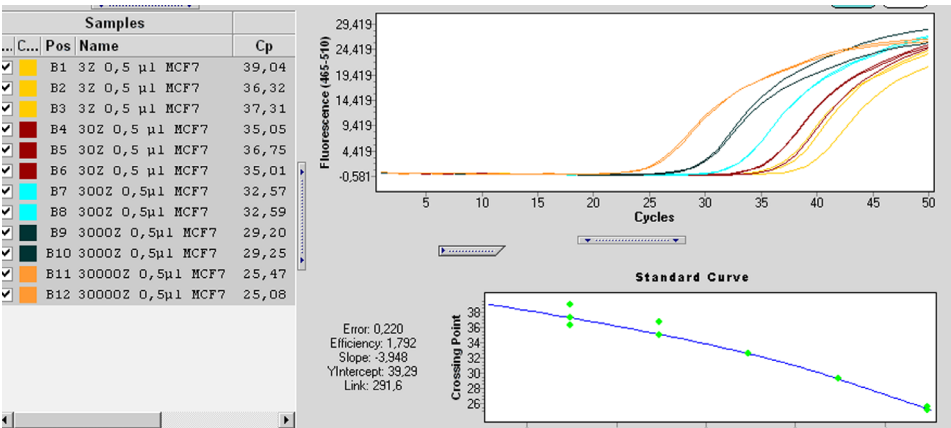


Fig. 2: RT-PCR analysis using the LightCycler® 480 Instrument of MCF7 cell lysate from 3 (far right) to 30,000 (far left) cells per well, targeting b2M. cDNA was synthesized from the MCF7 cell lysates using the Transcriptor cDNA Synthesis Kit. Detection of the PCR product was performed with Universal ProbeLibrary Probe #42 and primers specific for b2M.

The following results were obtained using the RealTime ready Cell Lysis Kit with MCF7 cells, followed by reverse transcription using the Transcriptor cDNA Synthesis Kit. Amplification and detection was performed on the LightCycler® 1536 Instrument with Universal ProbeLibrary Probe #42, primers specific for beta-2-microglobulin (b2M) and the RealTime ready DNA Probes Master.

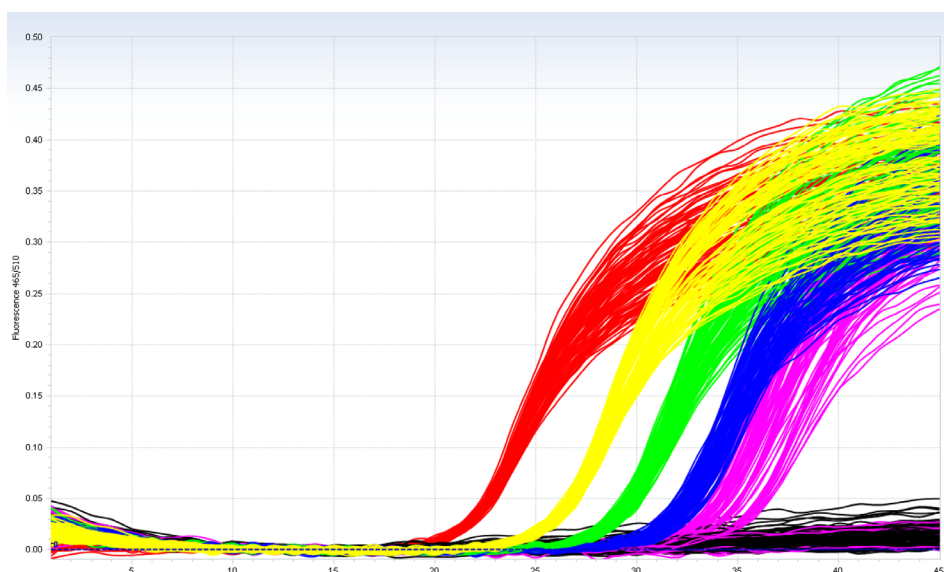


Fig. 3: RT-PCR analysis using the LightCycler® 1536 Instrument of MCF7 cell lysate from 3 to 30,000 cells per well, targeting b2M. cDNA was synthesized from the MCF7 cell lysates using the Transcriptor cDNA Synthesis Kit with a 1 µl total reaction volume. 20 cell equivalents (shown in red), 2 cell equivalents (shown in yellow), 0.2 cell equivalents (shown in green), 0.02 cell equivalents (shown in blue), 0.002 cell equivalents (shown in pink) and negative controls (shown in black) can be seen with 96 replicates per concentration.

4. Troubleshooting

Observation	Possible cause	Recommendation
Poor RT-PCR -performance	Cells were not washed with PBS, prior to the addition of the Cell Lysis Reagent.	It may be possible to omit the wash step if the growth medium has little or no serum.
	Too many cells	Use up to 10^5 cells per well. Seed up to 3×10^4 cells per well and grow overnight.
	RNA was degraded.	Wash with ice cold PBS before lysis.
		Keep lysate cold.
		After thawing the frozen lysate, continue immediately with cDNA synthesis.
		Do not store the lysate for more than 2 days at +2 to +8°C.
Late Cps	Cells were not attached to the bottom of the culture plate and removed by washing with PBS.	Allow cells to completely adhere to the plate by incubating longer, or harvest by isolating the supernatant and trypsinize the adherent cell fraction. Wash the combined cells with cold PBS.
	Too much PBS was left on the cells.	The PBS on the cells dilutes the Cell Lysis Reagent. Diluted Cell Lysis Reagent is not efficient in lysing and the RNases present in the samples are not completely inactivated.

5. Additional Information on this Product

5.1. Test Principle

How this Product Works

RealTime ready Cell Lysis Kit is an easy-to-use reagent for lysing cultured cells in 96- and 384-well plates, prior to reverse transcription. This product is designed specifically for gene expression assays using the Universal ProbeLibrary and a LightCycler® Master mix on the LightCycler® 480 System (96- or 384-well format), LightCycler® 96 Instrument or LightCycler® 1536 Instrument.

All that is required are the cells, RT-PCR reagents, primers and Universal ProbeLibrary probe.

Test Principle

The isolation of RNA from experimental samples is the first step in gene expression studies. RNA isolation can be a quite time-consuming and intensive process, especially when dealing with samples with small amounts of material/cells.

The RealTime ready Cell Lysis Kit is designed to quickly lyse cultured cells, prior to reverse transcription, without purifying the RNA, thus ensuring minimal loss of RNA and simplification of the whole gene expression workflow. The basic steps of using the RealTime ready Cell Lysis Kit and subsequent RT-PCR are:

1. Cultured cells, seeded in a 96-, or 384-well plate are washed with ice-cold PBS.
2. Cells are lysed by the addition of the Cell Lysis Reagent and incubation at room temperature. RNA is released from the lysed cells and protected from degradation, due to the presence of Protector RNase Inhibitor in the Cell Lysis Reagent.
3. Cell lysates are reverse transcribed into cDNA with Transcriptor Reverse Transcription. Genomic DNA from the cell lysate is optionally degraded by the addition of Thermolabile Nuclease (provided in the RealTime ready Cell Lysis Kit) to the reverse transcription reaction and a 10 min incubation at 29°C, prior to the reverse transcription step.
4. cDNA is then amplified using *e.g.* LightCycler® 480 Probes Master, or RealTime ready DNA Probes Master and the Universal ProbeLibrary.

5.2. Quality Control

The RealTime ready Cell Lysis Kit is tested to meet specifications of RT-qPCR.

6. Supplementary Information

6.1. 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>	
 Important Note: Information critical to the success of the current procedure or use of the product.	
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.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. 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.

Product	Pack Size	Cat. No.
Consumables		
LightCycler® 480 Multiwell Plate 96, white	5 x 10 plates	04 729 692 001
LightCycler® 480 Sealing Foil	50 foils	04 729 757 001
LightCycler® 480 Multiwell Plate 96, clear	5 x 10 plates	05 102 413 001
Instruments		
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
LightCycler® 96 Instrument	1 instrument	05 815 916 001
Reagents , kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
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
LightCycler® 480 RNA Master Hydrolysis Probes	1 kit, 5 x 100 reactions of 20 µl final volume each	04 991 885 001
Transcriptor Reverse Transcriptase	250 U, 25 reactions of 20 µl final volume	03 531 317 001
	500 U, 50 reactions of 20 µl final volume	03 531 295 001
	2,000 U, 4 x 500 U, 200 reactions of 20 µl final volume	03 531 287 001
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
Primer for cDNA Synthesis	40 µg	10 814 270 001
Primer "random"	2 mg, Primer, Random pd(N)6 Potassium Salt	11 034 731 001
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 High Fidelity cDNA Synthesis Kit	1 kit, 50 reactions, including 10 control reactions	05 081 955 001
	1 kit, 100 reactions	05 091 284 001
	1 kit, 200 reactions	05 081 963 001
Transcriptor Universal cDNA Master	1 kit, 100 reactions of 20µl final volume	05 893 151 001
LightCycler® 1536 DNA Probes Master	1 kit, 12,500 reactions, 2 µl each	05 502 381 001

6.4. Trademarks

LIGHTCYCLER is a trademark of Roche.

All third party product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to: <http://technical-support.roche.com>.

6.6. Regulatory Disclaimer

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

6.7. Safety Data Sheet

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

6.8. 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.

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support** Site.

Visit lifescience.roche.com, to download or request copies of the following **Materials**:

- Instructions for Use
- Safety Data Sheets
- Certificates of Analysis
- Information Material

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