INSTRUCTION MANUAL

Luna® Cell Ready Lysis Module

NEB #E3032S

Version 1.0_12/19

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Kit Components

The Luna Cell Ready Lysis Module (NEB #E3032) should be stored at –20°C upon receipt. If desired, the Luna Cell Ready Lysis Buffer (2X) can be stored at 4°C during ongoing use. This module has a shelf-life of 24 months when stored properly under these conditions.

Luna Cell Ready Lysis Module, NEB #E3032S, 100 reactions (50 µl)

Luna Cell Ready Lysis Buffer (2X)	2 x 1.4 ml
DNase I (RNase-free) (10X)	0.5 ml
Luna Cell Ready RNA Protection Reagent (25X)	0.25 ml
Luna Cell Ready Protease (25X)	0.2 ml
Luna Cell Ready Stop Solution (10X)	0.6 ml

Required Materials Not Included

Phosphate-buffered saline (PBS), chilled

Cells

Eppendorf tubes, PCR strip tubes, PCR plates

Pipettors and pipette tips (to minimize cross contamination, filter tips should be used)

Introduction:

The Luna Cell Ready Lysis Module (NEB# E3032) is part of the Luna Cell Ready One-Step RT-qPCR Kit. This module provides all the necessary components for preparation of cell lysates ready for One-Step RT-qPCR.

Coordinating the actions of DNase I and the Luna Cell Ready Protease, the Luna Cell Ready Lysis Module offers a simple workflow resulting in effective cell lysis, RNA release, and genomic DNA removal simultaneously in a 15-minute protocol. The Lysis Module includes a unique Luna Cell Ready RNA Protection Reagent that maintains RNA integrity during cell lysis. The lysis capacity spans 10–100,000 cells in a 50 µl lysis reaction. Up to 2 µl of lysate (equivalent to RNA from 0.2–4,000 cells) can be transferred into 20 µl downstream RT-qPCR reactions. In addition, the Luna Cell Ready Lysis Buffer contains a blue tracking dye, providing a visual indicator that can be followed throughout the entire reaction setup. This visible dye does not overlap spectrally with fluorophores commonly used in qPCR and does not interfere with real-time detection.

Cell lysates prepared from Luna Cell Ready Lysis Module have been tested for robust and sensitive RNA detection from cultured mammalian cells, including adherent cells, suspension cells, and cryopreserved cells.

For best results, the Luna Universal One-Step RT-qPCR Kit (NEB #E3005) or Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006) is recommended for dye-based or probe-based real-time quantitation of target RNA from the lysates.

For larger volume requirements, customized and bulk packaging is available through the NEB Customized Solutions department. Please contact NEBsolutions@neb.com for more information.

General Tips and Considerations

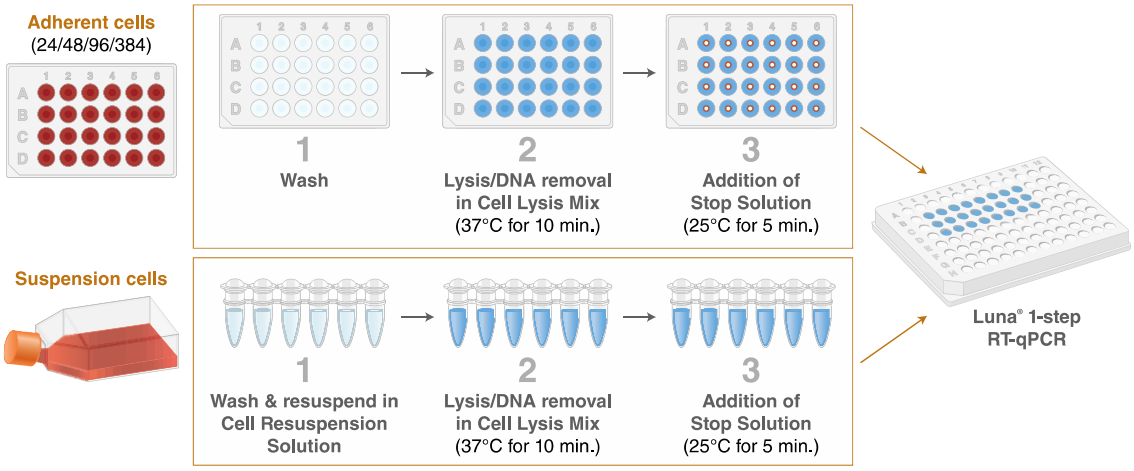
- General rules for cell culture should be followed.
- Intact RNA is essential for sensitive RT-qPCR detection. Apply precautions such as using filter tips, wearing gloves to avoid contamination, and minimizing sample handling time.
- To prevent undesirable bubbles from forming during cell lysis, use gentle pipetting and a brief spinning procedure. (Excess bubbles will prevent optimal cell lysis, causing low efficiency and inaccurate volume transfer during subsequent steps. After pipetting into the qPCR plate, if 1–2 small bubbles are present at the top of the well, the assay can proceed, as these bubbles will typically dissipate during the first denaturation step of the PCR.)
- The Luna Cell Ready Lysis Module has a lysis capacity of 10–100,000 cells in a 50 µl lysis reaction. Typically, 1–2 µl cell lysate (equivalent to RNA from 0.2–4,000 cells) can be transferred into a 20 µl downstream RT-qPCR. Most transcripts can be detected from 20 to 200 cells in a typical 20 µl reaction.
- The cell lysate can easily comprise up to 10% of the one-step RT-qPCR volume (e.g., 2 µl cell lysate in 20 µl RT-qPCR experiment).
- Cell lysates can be stored on ice up to five hours, at -20°C for up to five days and at -80°C for long term storage. Cell lysates are stable for up to five freeze and thaw procedures. Optimal results are seen using the lysates in downstream RT-qPCR experiments as soon as possible. Similarly, RT-qPCR reactions should proceed immediately after setup for best results.
- Some cell lines are difficult to lyse or may contain components inhibitory to RT-qPCR; therefore, several cell dilutions may be used to determine the appropriate range of cell numbers for lysis and RT-qPCR. To evaluate primers and targets for desirable linearity and efficiency, purified RNA controls should be used.
- During cell lysis, most genomic DNA is removed effectively. However, the use of RT-qPCR primers that span exons may further reduce qPCR signals from genomic DNA and therefore improve sensitivity.
- It is recommended to run all qPCR reactions in triplicate. This permits exclusion of outlier traces (e.g., due to unexpected plate issues, edge effects, or other problems) while maintaining accurate quantitation.
- When using multichannel pipettes, care should be taken to ensure consistency of pipetting volume.
- Primers purified with standard desalting methods are sufficient for use in Luna qPCR/RT-qPCR. In some cases, HPLC or PAGE purification may be helpful for assays that require increased sensitivity.

Luna Cell Ready Lysis Module Protocol

Before Use

- *Prepare cells in advance (various cell types such as adherent, suspension, or cryopreserved can be used) and ensure cells are intact before lysis.*
- *Consider testing several cell dilutions to determine the appropriate input range to use for lysis and RT-qPCR. Although a standard curve is not required for high throughput screening experiments, it is still recommended.*
- *Ensure that all components are thawed and mixed prior to use. Once thawed, place on ice prior to use.*

Workflow using Luna Cell Ready Probe One-Step RT-qPCR Kit.



Part I. Cell Lysate Preparation using Luna Cell Ready Lysis Module

Step 1. Processing Cells

Prepare Cell Resuspension Solution (CRS) by diluting Luna Cell Ready RNA Protection Reagent (25X) to 1X with cold PBS (e.g., for each sample, mix 2 µl Luna Cell Ready RNA Protection Reagent with 48 µl of 1X PBS to make 50 µl Cell Resuspension Solution). Cell Resuspension Solution is recommended for resuspending/diluting cells to reduce RNA damage during the handling process.

<table>
<tr><th>CELL CULTURE</th><th colspan="2">PROCEDURE</th></tr>
<tr><td>Adherent cells in 24/48/96/384 well plates</td><td colspan="2">1. Remove cell-culture medium.
2. Rinse briefly with cold PBS and aspirate PBS*</td></tr>
<tr><td>Adherent cells grow in other vessels</td><td>Detach cells using common sub-culturing technique</td><td rowspan="2">1. Transfer the desirable volume of cells and spin down the cell pellets
2. Rinse briefly with cold 1X PBS and aspirate PBS*</td></tr>
<tr><td>Suspension cells</td><td></td></tr>
<tr><td>Cryopreserved cells</td><td>Quickly thaw the cell stock at 37°C</td><td>3. Resuspend cells with CRS (up to 20,000 cells/µl). Store on ice and proceed to lysis within 10 mins.</td></tr>
</table>

* For high throughput screening (e.g., adherent cells in 96 wells or 384 well plates), cell wash is optional if medium removal can be completed via a plate flipping step.

Step 2. Prepare Cell Lysis Mix

1. Thaw Luna Cell Ready Lysis Buffer and Luna Cell Ready Stop Solution at room temperature, then place on ice with all other components. After thawing completely, briefly mix each component by inversion.
2. Prepare Cell Lysis Mix of all components, adding the Luna Cell Ready Protease immediately before use.

   Mix thoroughly by pipetting gently. Centrifuge briefly to collect the solution to the bottom of the tube and store on ice. For best results, the Cell Lysis Mix should be used immediately (**within 15 minutes)**.

COMPONENT	40 µl CELL LYSIS MIX	FINAL CONCENTRATION
Luna Cell Ready Lysis Buffer (2X)	25 µl	1X
DNase I (RNase-free) (10X)	5 µl	1X
Luna Cell Ready RNA Protection Reagent (25X)	2 µl	1X
Luna Cell Ready Protease (25X)	2 µl	1X
Nuclease-free Water	6 µl	

Up to 2,000 cells per µl lysis reaction is recommended. In a typical 50 µl lysis reaction, 100,000 cells can be lysed.

Step 3. Cell Lysis

For lysis using cell resuspension: mix up to 5 µl of cells with the Cell Lysis Mix to a final volume of 45 µl. Gently pipet up and down 6 times. Incubate the lysis reaction at 37°C for 10 min.*

For lysis in culture plates: aliquot an appropriate volume of Cell Lysis Mix into each well as indicated in the following table and incubate the reaction at 37°C for 10 min.*

For most efficient lysis, automatic shaking is recommended for cell densities higher than 200 cells/µl.

CULTURE PLATE	CELL LYSIS MIX
24 well	160 µl
48 well	80 µl
96 well	40 µl
384 well	8 µl

*Note: Lysis at room temperature is an option if cell density is less than 200 cells/µl in the lysate.

Step 4. Lysis Termination

Add 5 µl of Luna Cell Ready Stop Solution (10X) to the lysis reaction (45 µl) and mix well by pipetting up and down 6 times. Centrifuge briefly to collect the solution to the bottom of the tube. Incubate at 25°C for 5 min.

Cell lysates can be stored on ice up to five hours, at -20°C for up to five days and at -80°C for long term storage. Cell lysate is stable for up to five freeze and thaw procedures.

Part II. RNA Detection using Luna Universal One-Step RT-qPCR Kit or Probe RT-qPCR

The cell lysate can easily comprise up to 10% of the one-step RT-qPCR volume (i.e,. 2 µl cell lysate in 20 µl RT-qPCR experiment). Please refer to NEB #E3030 or NEB #E3031 manuals Part II for details.

Usage Notes:

Template Preparation and Concentration

The Luna Cell Ready Lysis Module has a lysis capacity of 10–100,000 cells in a 50 µl lysis reaction. Typically, 1–2 µl cell lysate (equivalent to RNA from 0.2–4,000 cells) can be transferred into a 20 µl downstream RT-qPCR). Most transcripts can be detected from 20 to 200 cells in a typical 20 µl reaction.

Troubleshooting Guide

OBSERVATION	POSSIBLE CAUSE(S)	SOLUTION(S)
Delayed qPCR traces or no amplification	Too many cells were used in the lysis reaction	• In general, up to 100,000 cells can be lysed successfully per 50 µl lysis reaction. If more cells are used, scale up the lysis reaction accordingly.
	Too few cells were used in the lysis reaction	• Increase cell numbers up to 2,000 per µl lysate for rare transcripts
	Excess amount of culture medium or PBS remained (e.g., during high throughput screening)	• Remove medium/PBS as thoroughly as possible by good aspiration or plate-flip techniques. PBS carryover should be ≤ 10% of total lysis reaction volume.
	Components in the lysis reaction are not fully inactivated by stop solution or excessive stop solution was used	• Add stop solution to the lysis reaction and mix well • Use the recommended volume of Luna Cell Ready Stop Solution
	Some cell lines may contain high levels of inhibitors for cell lysis or RT-qPCR	• The optimal input cell number for lysis may differ for different cell lines or culture conditions. Please refer to the kit FAQs at www.neb.com/E3030 for the most up-to-date list of cell lines/cells tested. • Try to reduce cell input up to 100-fold
	RNA was degraded during harvest, wash or cell lysis	• Ensure cells are intact prior to lysis • Include Luna Cell Ready RNA Protection Reagent for cell resuspension and dilution • Proceed to RT-qPCR immediately after lysis
	RNA was degraded during lysate storage	• In general, cell lysates should be kept on ice no more than five hours or at -20°C for up to five days. For longer storage, -80°C is recommended. • Lysates can typically tolerate up to five freeze and thaw cycles • For best result, cell lysates containing < 2 cells/µl should be used immediately
	Cell lysis is not efficient, RNA is not fully released	• Use recommended amount of Luna Cell Ready Protease • Use 37°C for cell lysis. Lysis time can be extended up to 20 minutes, if needed • Reduce cell input to 100-fold
	The sample does not contain the target RNA	• Verify RT-qPCR detection using purified RNA • If positive RNA control is available, this can be spiked into the cell lysate to confirm detection

OBSERVATION	POSSIBLE CAUSE(S)	SOLUTION(S)
Standard curve using cell dilutions has a poor correlation coefficient or undesirable efficiency (outside of 90%–110%)	Reaction conditions are incorrect or cycling protocol is incorrect	• Verify that all steps of the protocol were followed correctly • Refer to the proper RT-qPCR cycling protocol in this user manual. Use a 55°C RT step temperature. For ABI instruments, use a 1 minute for 60°C annealing/extension step.
	Inaccurate pipettes or inaccurate serial dilutions of cells	• Ensure pipettes are calibrated regularly and use proper pipetting techniques • Cell resuspension is heterogeneous; mix well before pipetting
	High level of inhibitors from cellular components cause efficiency above 110%	• Reduce the input cell number up to 100-fold • Shaking plates during in-well lysis may lead to more effective lysis
	RNA was degraded during cell lysis or RT-qPCR setup	• Avoid exposing lysates to room temperature after lysis • Assay lysate as soon as possible • RT-qPCR should proceed immediately after setup for best results. (Store at 4°C for no more than 5 hours).
	Threshold is improperly set for the qPCR traces	• Verify the threshold is set in the exponential region of qPCR traces
Inconsistent qPCR traces for triplicate data	Improper pipetting during RT-qPCR assay set-up	• Cell lysates contain detergents; pay attention to ensure accurate pipetting (e.g., no leftover in the pipette tip)
	qPCR plate film has lost its seal, causing evaporation and different fluorescence values	• Ensure the qPCR plate is properly sealed • Exclude problematic trace(s) from data analysis
	Poor mixing of reagents during RT-qPCR set-up or bubbles cause an abnormal qPCR trace	• Ensure thorough mixing of reagents after thawing • Centrifuge the qPCR plate after setup • Exclude outlier trace(s) from data analysis
Signal in the No-RT control	Incomplete genomic DNA digestion	• Use the recommended amount of DNase I and Luna Cell Ready Protease • Mix lysis reaction during 37°C incubation • Reduce cell input up to 100-fold
	Cross contamination from RT-qPCR products	• Avoid opening RT-qPCR reactions • Perform UDG treatment
Further questions related to RT-qPCR		• Refer to the troubleshooting and FAQ's sections of Luna Universal One-Step RT-qPCR kit at www.neb.com/E3005 and of Luna Universal Probe One-Step RT-qPCR Kit at www.neb.com/E3006

Ordering Information

NEB #	PRODUCT	SIZE
E3032S	Luna Cell Ready Lysis Module	100 reactions
COMPANION PRODUCTS		
E3030S	Luna Cell Ready One-Step RT-qPCR	100 reactions
E3031S	Luna Cell Ready Probe One-Step RT-qPCR Kit	100 reactions
E3005S/L	Luna Universal One-Step RT-qPCR Kit	200/500 reactions
E3005X	Luna Universal One-Step RT-qPCR Kit	1,000 reactions
E3005E	Luna Universal One-Step RT-qPCR Kit	2,500 reactions
E3006S/L	Luna Universal Probe One-Step RT-qPCR Kit	200/500 reactions
E3006X	Luna Universal Probe One-Step RT-qPCR Kit	1,000 reactions
E3006E	Luna Universal Probe One-Step RT-qPCR Kit	2,500 reactions

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