INSTRUCTION MANUAL

NEBNext® Single Cell Lysis Module

NEB #E5530S

96 reactions

Version 4.0_02/24

Table of Contents

The NEBNext Single Cell Lysis Module Includes

The volumes provided are sufficient for preparation of up to 96 reactions (NEB #E5530S). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (white) Murine RNase Inhibitor
- (white) NEBNext Cell Lysis Buffer

The NEBNext Single Cell Lysis Module is Designed for use with the Following

NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina® (NEB #E6420)

NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module (NEB #E6421)

Required Materials Not Included

- Nuclease-free Water
- DNA LoBind Tubes (Eppendorf® #022431021) or DNase RNase free PCR strip tubes (USA Scientific 1402-1708)
- Thermal cycler
- Microcentrifuge

Applications

The NEBNext Single Cell Lysis Module contains the enzymes and buffers required to lyse isolated cultured and primary cells to extract RNA. The module is optimized for use with the NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina (NEB #E6420) or NEBNext Single Cell/Low Input cDNA Synthesis and Amplification Module (NEB #E6421). The fast, user-friendly workflow also has minimal hands-on time.

Each kit component must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together by construction of indexed libraries made from single cells and sequenced on an Illumina sequencing platform.

For larger volume requirements, customized and bulk packaging is available by purchasing through the Customized Solutions Team at NEB. Please contact custom@neb.com for further information.

Protocol for Cells: Cell Lysis

Symbols

This is a point where you can safely stop the protocol and store the samples prior to proceeding to the next step in the protocol.

This caution sign signifies a step in the protocol that has two paths leading to the same end point but is dependent on a user variable, like the type of input.

- *Colored bullets indicate the cap color of the reagent to be added.*

Sample Recommendations

This protocol is intended for isolated cultured or primary cells, but is not compatible with fixed cells.

Cells should be intact and sorted into cold cell lysis buffer provided in the module. See Section 1.2 and 1.3 for cell lysis buffer dilution and recommended volumes before use. Cells should be washed and resuspended in PBS prior to isolation/sorting, because carryover of media may affect the cDNA synthesis efficiency.

Starting Material

Isolated single, tens or hundred cells.

Notes

Keep all buffers and enzymes on ice, unless otherwise indicated.

1.1. Sample and Reagents Preparation

1.1.1. Briefly centrifuge the tube containing Murine RNase Inhibitor to collect solutions to the bottom of the tubes, then place on ice.

1.1.2. Thaw the (10X) NEBNext Cell Lysis Buffer at room temperature. If it appears cloudy after thawing, incubate briefly at 37°C to clear up the solution. Mix and centrifuge briefly to collect the solution to the bottom of the tube. Leave the (10X) NEBNext Cell Lysis Buffer bottle at 4°C or room temperature for storage.

1.2. Cell Collection (< 1 µl volume) and Lysis

If the PBS carryover volume from cell isolation/sorting is < 1 µl, cells can be dispensed directly into cold 1X NEBNext Cell Lysis Buffer (without accounting for added volume). If PBS carryover volume from cell isolation/sorting is ≥ 1 µl, skip to Section 1.3.

1.2.1. On ice prepare 1X NEBNext Cell Lysis Buffer in an RNase-free tube as follows:

COMPONENT	VOLUME (µl) PER REACTION
○ (white) NEBNext Cell Lysis Buffer (10X)	0.5 µl
○ (white) Murine RNase Inhibitor	0.25 µl
Nuclease-free Water	4.25 µl
Total Volume	**5 µl**

1.2.2. Mix solution thoroughly by pipetting, avoiding bubbles. Centrifuge briefly to collect solution to the bottom of the tube.

1.2.3. Dispense cells directly into cold 5 µl 1X Cell Lysis Buffer. After dispensing, cells can be flash-frozen and stored at -80°C for future use.

Cells can also be lysed as outlined in Step 1.2.4. In that case, proceed to the next step.

1.2.4. Incubate at room temperature for 5 minutes to lyse cells. Proceed immediately to cDNA synthesis and amplification using NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina (NEB #E6420) or NEBNext Single Cell/Low Input cDNA Synthesis and Amplification Module (NEB #E6421).

1.3. Cell Collection (≥ 1 µl volume) and Lysis

1.3.1. If the PBS carryover volume from cell isolation/sorting is ≥ 1 µl or the cells have already been collected in a solution with a volume ≥ 1 µl, prepare a 1X Cell Lysis Buffer on ice according to the table below, accounting for the carryover cell volume.

REAGENT	VOLUME (µl) PER REACTION
Carryover Cell Volume (in PBS)	1–5 µl
○ (white) NEBNext Cell Lysis Buffer (10X)	0.8 µl
○ (white) Murine RNase Inhibitor	0.4 µl
Nuclease-free Water	Variable (based on carryover cell volume)
Total Volume	**8 µl**

1.3.2 Add prepared cell lysis buffer to the cells, mix solution thoroughly by pipetting, avoiding bubbles. Centrifuge briefly to collect solution to the bottom of the tube.

Cells can be flash frozen and stored at -80°C for future use or lysed as outlined in step 1.3.3.

1.3.3. Incubate at room temperature for 5 minutes to lyse cells.

Proceed immediately to cDNA synthesis and amplification using NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina (NEB #E6420) or NEBNext Single Cell/Low Input cDNA Synthesis and Amplification Module (NEB #E6421).

Please read the FAQ section on NEB.com for additional information about this product. Please read the FAQ section on NEB.com for additional information about this product.

Kit Components

NEB #E5530S Table of Components

NEB #	PRODUCT	VOLUME
E6429AA	Murine RNase Inhibitor	0.096 ml
E6428AA	NEBNext Cell Lysis Buffer	0.192 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	9/18
2.0	Applied new manual format.	2/20
3.0	Component name updates and new table format.	12/22
4.0	Updated Safe Stop information as well as table formatting and header/footer and legal footer.	02/24