

NEBNext[®] RNA First Strand Synthesis Module

NEB #E7525S/L

24/96 reactions

Version 6.0_7/22

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The NEBNext RNA First Strand Synthesis Module Includes

The volumes provided are sufficient for preparation of up to 24 reactions (NEB #E7525S) and 96 reactions (NEB #E7525L). All reagents should be stored at –20°C. Colored bullets represent the color of the cap of the tube containing the reagent.

- (pink) NEBNext First Strand Synthesis Reaction Buffer
- (pink) Random Primers
- (pink) ProtoScript II Reverse Transcriptase
- (pink) Murine RNase Inhibitor

The NEBNext RNA First Strand Synthesis Module is Designed for use with the Following:

- NEBNext Ultra[™] II Non-Directional RNA Second Strand Synthesis Module (NEB #E6111)
- NEBNext Ultra End Repair/dA-Tailing Module (NEB #E7442)
- NEBNext Ultra Ligation Module (NEB #E7445)
- NEBNext Q5[®] Hot Start HiFi PCR Master Mix (NEB #M0543)

Required Materials Not Included

- PCR machine
- Tempassure PCR flex-free 8-tube strips (USA Scientific #1402-4708)

Applications

The NEBNext RNA First Strand Synthesis Module contains enzymes and buffers required to convert a broad range of input amounts of RNA into cDNA using random priming. The fast, user-friendly workflow has minimal hands-on time and is compatible with upstream Poly(A) mRNA enrichment and rRNA depletion methods; it is also compatible with downstream second strand cDNA synthesis for both directional and non-directional RNA-seq workflows. Single stranded cDNA can be used directly for second strand cDNA synthesis.

Each module component must pass rigorous quality control standards, and for each new lot the entire set of reagents is functionally validated together with NEB #E6111 or NEB #E7550, #E7445, #E7442, and #M0543 to construct an indexed transcriptome library that is sequenced on an Illumina sequencing platform.

For larger volume requirements, customized and bulk packaging is available by purchasing through the OEM/Bulks department at NEB. Please contact OEM@neb.com for further information.

Protocol

Symbols



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 RNA input.



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.



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

Note: This protocol is for total RNA, purified mRNA or rRNA depleted RNA only. For use with the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490) or the NEBNext RNA Depletion Kits and the NEBNext Ultra II Directional RNA Library Prep Workflow, please follow the protocol in the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760) manual.

Protocol for use with Previously Fragmented RNA

Starting Material: 100–250 ng of purified mRNA fragmented to 200 nt and cleaned up in 13.5 μ l of nuclease free water.

1. First Strand cDNA Synthesis (Non-directional Reaction Setup). Mix the following components in a sterile PCR tube:

COMPONENT	VOLUME
Fragmented mRNA	13.5 μ l
Random Primers	1 μ l
Total Volume	14.5 μ l

2. Incubate in a preheated thermal cycler for 5 minutes at 65°C with heated lid set to 105°C. Hold at 4°C.
3. Spin tube briefly and place on ice
4. To the fragmented mRNA and Random Primers add:

COMPONENT	VOLUME
First Strand Synthesis Reaction Buffer	4 μ l
Murine RNase Inhibitor	0.5 μ l
Total Volume	19 μ l

5. Incubate in a preheated thermal cycler for 2 minutes at 25°C.
6. Add 1 μ l ProtoScript II Reverse Transcriptase to the reaction.
7. Incubate the samples in a preheated thermal cycler (with the heated lid set to $\geq 80^\circ\text{C}$)
 - 10 minutes at 25°C
 - 50 minutes at 42°C
 - 15 minutes at 70°C
 - Hold at 4°C
8. Place the tube on ice.
9. Proceed directly to second strand synthesis using NEBNext mRNA Second Strand Synthesis Module (NEB #E6111).

Kit Components

NEB #E7525S Table of Components

NEB #	PRODUCT	VOLUME
E7421A	NEBNext First Strand Synthesis Reaction Buffer	0.192 ml
E7422A	Random Primers	0.048 ml
E7423A	ProtoScript II Reverse Transcriptase	0.024 ml
E7424A	Murine RNase Inhibitor	0.015 ml

NEB #E7525L Table of Components

NEB #	PRODUCT	VOLUME
E7421AA	NEBNext First Strand Synthesis Reaction Buffer	0.768 ml
E7422AA	Random Primers	0.192 ml
E7423AA	ProtoScript II Reverse Transcriptase	0.096 ml
E7424AA	Murine RNase Inhibitor	0.048 ml

Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	
2.0		3/13
3.0	Create "Kit Component – Table of Components" for small and large size kits. Delete individual component information pages.	4/18
4.0	Add the list of "Designed for Use with the Following". Update protocols.	12/18
5.0	New Format Applied.	3/20
6.0	Update protocol	7/22

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