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SPECIAL PRICES until 30.06.2022
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Minding your caps and tails – considerations for functional mRNA synthesis

Applications of synthetic mRNA have grown and become considerably diversified in recent years. Examples include the generation of pluripotent stem cells (1-3), vaccines and therapeutics (4-6), and CRISPR/Cas9 genome editing applications (7-9). The basic requirements for a functional mRNA – a 7-methylguanylate cap at the 5’ end and a poly(A) tail at the 3’ end – must be added in order to obtain efficient translation in eukaryotic cells. Additional considerations can include the incorporation of internal modified bases, modified cap structures and polyadenylation strategies. Strategies for in vitro synthesis of mRNA vary according to the desired scale of synthesis.

A nascent mRNA, synthesized in the nucleus, undergoes modifications before it can be translated into proteins in the cytoplasm. For a mRNA to be functional, it requires modified 5’ and 3’ ends and a coding region (i.e., an open reading frame (ORF) encoding the protein of interest) flanked by the untranslated regions (UTRs). The nascent mRNA (pre-mRNA) undergoes two significant modifications in addition to splicing. During synthesis, a 7-methylguanylate structure, also known as a “cap”, is added to the 5’ end of the pre-mRNA, via 5’ → 5’ triphosphate linkage. This cap protects the mature mRNA from degradation, and also serves a role in nuclear export and efficient translation.

The second modification occurs post-transcriptionally at the 3’ end of the nascent RNA molecule, and is characterized by addition of approximately 200 adenylate residues (poly(A) tail). This formation is essential for the survival and function of a nascent mRNA, containing the information needed for translation into proteins.

Transcription for enzyme-based capping (post-transcriptional capping)

Standard RNA synthesis reactions produce the highest yield of RNA transcript (typically ≥100 µg per 20 µl in a 1 hr reaction using the HiScribe Quick T7 High Yield RNA Synthesis Kit, NEB #E2050S). Transcription reactions are highly scalable.

Following transcription, the RNA is treated with DNase I (NEB #M0303) to remove the DNA template, and purified using an appropriate column, kit or magnetic beads, prior to capping. This method produces high yields of RNA with 5’ triphosphate termini that must be converted to cap structures. In the absence of template-encoded poly(A) tails, transcripts produced using this method bear 3’-termini that also must be polyadenylated in a separate enzymatic step, as described below in “Post-transcriptional capping and Cap-1 methylation”.

Transcription with dinucleotide co-transcriptional capping

In co-transcriptional capping, a cap analog is introduced into the transcription reaction, along with the four standard nucleotide triphosphates, in an optimized ratio of cap analog to GTP 4:1. This allows initiation of the transcript with the cap structure in a large proportion of the synthesized RNA molecules. This approach produces a mixture of transcripts, of which ~80% are capped, and the remainder have 5’-triphosphate ends. Decreased overall yield of RNA products results from the lower concentration of GTP in the reaction.

There are several cap analogs used in co-transcriptional RNA capping (3). The most common are the standard 7-methyl guanosine (m7G) cap analog and anti-reverse cap analog (ARCA), also known as 3’-O-me 7-meGuGpppG cap analog. ARCA is methylated at the 3’ position of the m7G, preventing RNA elongation by phosphodiester bond formation at this position. Thus, transcripts synthesized using ARCA contain 5’-m7G cap structures in the correct orientation, with the 7-methylated G as the terminal residue. In contrast, the m7G cap analog can be incorporated in either the correct or the reverse orientation.

Transcription with CleanCap Reagent AG co-transcriptional capping

The use of CleanCap reagent AG results in significant advantages over traditional dinucleotide co-transcriptional capping. CleanCap Reagent AG is a trinucleotide with a 5’-m7G joined by a 5’ → 5’ triphosphate linkage to an AG sequence. The adenine has a methyl group on the 2’-O position. The incorporation of this trinucleotide in the beginning of a transcript results in a Cap-1 structure.

In order to use CleanCap Reagent AG in an in vitro transcription reaction the template must contain an AG in place of a GG following the T7 promoter in the initiation sequence. Unlike traditional co-transcriptional capping, reduction of GTP concentration is not required and therefore yield is higher and high capping efficiencies, >95%, are achieved.

Transcription with complete substitution with modified nucleotides

RNA synthesis can be carried out with a mixture of modified nucleotides in place of the regular mixture of A, G, C and U triphosphates. For expression applications, the modified nucleotides of choice are the naturally occurring 5’-methylycytidine and/or pseudouridine in the place of C and U, respectively. These have been demonstrated to confer desirable properties to the mRNA, such as increased mRNA stability, increased translation, and reduced immune response in the key applications of protein replacement and stem-cell differentiation (1). It is important to note that nucleotide choice can influence the overall yield of mRNA synthesis reactions.

Fully substituted RNA synthesis can be achieved using the HiScribe T7 mRNA Kit with CleanCap Reagent AG (NEB #E2080). HiScribe T7 High-Yield RNA Synthesis Kit (NEB #E2040) or HiScribe SP6 RNA Synthesis Kit (NEB #E2070) in conjunction with NTPs with the desired modification. Transcripts made with complete replacement of one or more nucleotides may be post-transcriptionally capped (see next section), or may be co-transcriptionally capped by including...
Figure 1: *In vitro* transcription options based upon capping strategy

**POST-TRANSCRIPTIONAL ENZYME-BASED mRNA CAPPING**

With Vaccinia Capping System

- RNA polymerase promoter
- DNA template
- Phosphate
- RNA polymerase + NTPs
- Vaccinia Capping Enzyme
- mRNA transcript
- SAM
- GTP
- Cap-0 mRNA

With Vaccinia Capping System and mRNA Cap 2´-O-Methyltransferase

- RNA polymerase promoter
- DNA template
- 5´-Phosphate
- RNA polymerase + NTPs
- Vaccinia Capping Enzyme
- mRNA transcript
- SAM
- GTP
- mRNA Cap
- Cap-1 mRNA

**CO-TRANSCRIPTIONAL mRNA CAPPING**

With Anti-Reverse Cap Analog (ARCA)

- RNA polymerase promoter
- DNA template
- OCH₃
- ARCA
- mGpGpG
- RNA polymerase
- NTPs
- Cap-0 mRNA

With CleanCap Reagent AG

- RNA polymerase promoter
- DNA template
- OCH₃
- CleanCap Reagent AG
- mGpGpA
- RNA polymerase
- NTPs
- Cap-1 mRNA

Enzyme-based capping (top) is performed after *in vitro* transcription using 5´-triphosphate RNA, GTP, and S-adenosyl- methionine (SAM). Cap-0 mRNA can be converted to Cap-1 mRNA using mRNA cap 2´-O-methyltransferase (MTase) and SAM in a subsequent or concurrent reaction. The methyl group transferred by the MTase to the 2´-O of the first nucleotide of the transcript is indicated in red. Conversion of ~100% of 5´-triphosphorylated transcripts to capped mRNA is routinely achievable using enzyme-based capping.

Co-transcriptional capping (bottom) uses an mRNA cap analog, shown in yellow, in the transcription reaction. For ARCA (anti-reverse cap analog) (left), the cap analog is incorporated as the first nucleotide of the transcript. ARCA contains an additional 3´-O-methyl group on the 7-methylguanosine to ensure incorporation in the correct orientation. The 3´-O-methyl modification does not occur in natural mRNA caps. Compared to reactions not containing cap analog, transcription yields are lower. ARCA-capped mRNA can be converted to cap 1 mRNA using mRNA cap 2´-OMTase and SAM in a subsequent reaction. CleanCap Reagent AG (right) uses a truncated cap analog that requires a modified template initiation sequence. A natural Cap-1 structure is accomplished in a co-transcriptional reaction.

"GMP-grade" is a branding term NEB uses to describe reagents manufactured at NEB’s Rowley facility. The Rowley facility was designed to manufacture reagents under more rigorous infrastructure and process control to achieve more stringent product specifications and customer requirements. Reagents manufactured at NEB’s Rowley facility are manufactured in compliance with ISO 9001 and ISO 13485 quality management system standards. However, at this time, NEB does not manufacture or sell products known as Active Pharmaceutical Ingredients (APIs), nor does NEB manufacture its products in compliance with all of the Current Good Manufacturing Practice regulations.

A-tailing using *E. coli* Poly(A) Polymerase

The poly(A) tail confers stability to the mRNA and enhances translation efficiency. The poly(A) tail can be encoded in the DNA template by using an appropriately tailed PCR primer, or it can be added to the RNA by enzymatic treatment with *E. coli* Poly(A) Polymerase (NEB #M0276). The length of the added tail can be adjusted by titrating the Poly(A) Polymerase in the reaction.

For mRNA synthesis from templates with encoded poly(A) tails, the HiScribe T7 ARCA mRNA Synthesis Kit (NEB #E2065) provides an optimized formulation for co-transcriptionally capped transcripts.

In summary, when choosing the right workflow for your functional mRNA synthesis needs, you must balance your experimental requirements for the mRNA (e.g., internal modified nucleotides) with scalability (i.e., ease-of-reaction setup vs. yield of final product).

Products from NEB are available for each step of the RNA synthesis workflow: GMP-grade® reagents suitable for the large scale manufacture of therapeutics mRNA are available through our Customized Solution Group.
New tools for your (m)RNA Synthesis (IVT)

New Products:

**HiScribe™ T7 mRNA Kit with CleanCap® Reagent AG**

The HiScribe T7 mRNA Kit with CleanCap Reagent AG utilizes an optimized RNA synthesis formulation and trinucleotide cap analog technology to co-transcriptionally cap mRNAs containing a natural Cap-1 structure in a single simplified reaction without compromising RNA yield. Using a DNA template with a T7 promoter sequence followed by an AG initiation sequence and an encoded poly(A) tail, mRNAs can be transcribed with a 5’-m7G Cap-1 structure that is polyadenylated, translationally competent and able to evade the cellular innate immune response.

**Advantages:**

- Synthesize and cap mRNA in a single reaction
- Evade immune response with natural Cap-1 structure
- Generate high yields of mRNA, up to 1.8 mg per kit
- Suitable for full or partial modified nucleotide substitutions

**Comparison of RNA Yields from in vitro Reagent AG Transcription Reactions with no cap analog, ARCA, or CleanCap Reagent AG**

All reactions were performed with 5 mM CTP, 5 mM UTP and 6 mM ATP. Standard IVT reactions included 5 mM GTP and no cap analog. ARCA reactions contained a 4:1 ratio of ARCA:GTP (4mM:1mM). IVT with CleanCap Reagent AG contained 5 mM GTP and 4 mM CleanCap Reagent AG and was performed as described (Standard mRNA Synthesis, HiScribe T7 mRNA Kit with CleanCap Reagent AG). Reactions were incubated for 2 hours at 37°C, purified and quantified by NanoDrop.

**Ordering information:**

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<td>HiScribe T7 mRNA Kit with CleanCap Reagent AG</td>
<td>E2080S</td>
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Also available:

**HiScribe™ T7 (Quick) High Yield RNA Synthesis Kit**

The HiScribe T7 High Yield RNA Synthesis Kit (NEB #E2040) delivers robust high yield RNA synthesis (up to 180 μg/reaction) for a wide range of template sizes. Flexible protocols ensure that performance is maintained even under demanding conditions, such as extended reaction time using very low amounts of template. Protocols are included for partial or complete incorporation of modified or labeled nucleotides in the transcript body, and cap analogs at the RNA 5’ end.

The HiScribe T7 Quick High Yield RNA Synthesis Kit (NEB #E2050) utilizes a master mix format, allowing for faster reaction setup. DNase I and lithium chloride are included for DNA template removal and quick RNA purification.

**Advantages:**

- Streamlined format & Quick Workflows
- High Yield – up to 180 μg of RNA from a standard 20 μl reaction
- Flexibility – enables incorporation of cap analogs, radiolabeled and modified nucleotides

**Ordering information:**

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<tr>
<td>HiScribe SP6 RNA Synthesis Kit</td>
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Hi-T7® RNA Polymerase for reduced dsRNA by-product formation

Hi-T7 RNA Polymerase is an engineered DNA-dependent RNA polymerase that is highly specific for T7 phage promoters, designed for *in vitro* transcription of RNA at higher temperatures and recommended for experienced users interested in building and optimizing their own *in vitro* transcription reactions.

**Advantages:**
- Active from 37-56°C, optimal incubation temperature is 50-52°C.
- Increased co-transcriptional capping efficiency with cap analogs
- Decreased unwanted immunogenicity from RNA synthesized at higher temperature due to reduced dsRNA by-product formation

**Immunoblot using an anti-dsRNA antibody (J2) shows presence of dsRNA by-products in the IVT reactions for both T7 and Hi-T7 RNA Polymerases when IVT is performed at 37°C. HPLC purification of the IVT RNA eliminates dsRNA by-products. dsRNA by-products are reduced when IVT is performed at 50°C (or higher temperatures) with Hi-T7.**

**Ordering information:**

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<tr>
<td>Hi-T7 RNA Polymerase (High Concentration)</td>
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**Explore our interactive timeline of mRNA discoveries**

Get caught up on key mRNA discoveries with our interactive timeline, featuring a selection of publications and resources from the last seven decades.

**Discover these RNA Synthesis resources at [www.NEBrna.com](http://www.NEBrna.com)**

**Request from your local distributor:**
- RNA Technical Guide – Find in depth information on tools designed to streamline your RNA workflows
- RNA Synthesis Brochure – Learn more about NEB’s products for RNA synthesis, which range from template generation to poly(A) tailing
- GMP-Grade* Reagents for RNA Synthesis Brochure – Learn about the benefits of GMP-grade materials available from NEB, and how they can be used in your mRNA synthesis workflow
NEBuilder® HiFi DNA Assembly – reformulated for improved performance

Assembling DNA fragments is a key part of both synthetic biology techniques and cloning. NEBuilder HiFi DNA Assembly enables virtually error-free joining of DNA fragments, even those with 5’- and 3’-end mismatches. This flexible kit enables simple and fast seamless cloning utilizing a proprietary high-fidelity polymerase. Find out why NEBuilder HiFi is the next generation of DNA assembly and cloning.

Advantages:
- Seamless and ordered HiFi cloning in as little as 15 minutes
- “Standard-size” cloning and larger gene assembly products, up to 11 fragments
- No licensing fees from NEB for NEBuilder products

For more information, visit NEBuilderHiFi.com.

NEBridge™ Ligase Master Mix

Offering flexibility and convenience for users, NEBridge Ligase Master Mix performs high efficiency and high-fidelity Golden Gate Assembly with a broad range of NEB Type IIS restriction enzymes.

NEBridge Ligase Master Mix is a 3X master mix for Golden Gate Assembly. Designed for use with NEB Type IIS restriction enzymes, this master mix contains T4 DNA Ligase in an optimized reaction buffer with a proprietary ligation enhancer. Users only need to choose their preferred NEB Type IIS restriction enzyme and add DNA substrates to be assembled. Low complexity single-fragment insertions, as well as moderate complexity (3–6 fragment) and high complexity (7–25+ fragment) assemblies, are all supported with this optimized reagent and accompanying protocols.

Advantages:
- Optimized for efficient and accurate Golden Gate Assembly
- Use with NEB Type IIS restriction enzymes
- Use for seamless cloning – no scar remains following assembly
- Ideal for ordered assembly of multiple fragments (2–25+) in a single reaction
- Can also be used for cloning single inserts and library construction
- Design primers with our free tool available at GoldenGate.neb.com

For more information, visit NEBridge.com.

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<td>NEBuilder HiFi DNA Assembly Cloning Kit</td>
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<td>NEBuilder HiFi DNA Assembly Bundle for Large Fragments</td>
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To learn more, please visit www.neb.com/M1100

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<td>NEBridge Ligase Master Mix</td>
<td>M1100S</td>
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Try NEB’s competent cells for higher efficiency cloning

NEB’s growing line of competent cells includes several popular strains for cloning. Our cloning strains include derivatives of the industry standards, DH5α and DH10B. NEB Turbo is unique to NEB and allows colony growth after 6.5 hours. NEB’s dam–/dcm– strain enables isolation of plasmids free of Dam and Dcm methylation. NEB Stable is recommended in all difficult cloning experiments. Our cells are all extensively tested for phage resistance, antibiotic resistance and sensitivity, blue/white screening and transformation efficiency. High efficiency, 5 minute transformation and electroporation protocols are provided, when applicable.

Benefit from high transformation efficiencies

Transformation efficiencies were compared using manufacturers’ recommended protocols. Values shown are the average of triplicate experiments.

Benefit from our Special Price Campaign on all Cloning Strains*

<table>
<thead>
<tr>
<th>FEATURES</th>
<th>NEB S-alpha Competent E. coli (NEB #C2987)</th>
<th>NEB Turbo Competent E. coli (NEB #C2894)</th>
<th>NEB S-alpha F’ Competent E. coli (NEB #C2985)</th>
<th>NEB 10-beta Competent E. coli (NEB #C3019)</th>
<th>dam–/dcm– Competent E. coli (NEB #C2915)</th>
<th>NEB Stable Competent E. coli (NEB #C2040)</th>
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<td>Versatile</td>
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<td>Fast growth (&lt; 8 hours)</td>
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<td>Toxic gene cloning</td>
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<td>Large plasmid/BAC cloning</td>
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<td>Dam/Dcm-free plasmids</td>
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**FEATURES**

- Versatile
- Fast growth (< 8 hours)
- Toxic gene cloning
- Large plasmid/BAC cloning
- Dam/Dcm-free plasmids
- Retroviral/lentiviral vector cloning
- RecA-

**FORMATS**

- Chemically competent
- Electrocompetent
- Subcloning
- 96-well plate format
- 384-well plate format
- 8-tube strips

Advantages:

- High transformation efficiencies
- Compatible with NEBuilder HiFi DNA Assembly and NEBridge Golden Gate Assembly reactions, as well as ligation reactions. No dilution required!
- All strains are free of animal products and are T1 phage resistant
- Outgrowth medium and control plasmid are included
- Choose from a variety of convenient formats, including single-use tubes
- Bulk formats and custom packaging are available

For a complete list of available different formats, please visit [www.neb.com/CloningCompCells](http://www.neb.com/CloningCompCells)

Request new free Tech Guide "Molecular Cloning" and the Competent Cells brochure from your local distributor!

*Get special prices on all NEB competent cells for cloning as well as the Quick Load Purple DNA Ladders (page 12). Campaign ends June 30th, 2022. Please ask your local distributor for details.
LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG – lyophilized to be stored & shipped at room temperature

With the point-of-care market becoming more focused on the development of robust, accurate and cost-effective diagnostic tests for use outside of traditional hospital and laboratory settings, there is a growing need for reagents that can withstand ambient shipping and storage. Lyophilization is the preferred solution and is a well-established technology across a number of industries.

Bringing together expertise in enzyme development, manufacturing and lyophilization, NEB and Fluorogenics Limited have created shelf-stable, lyophilized products that do not sacrifice the high-performance qualities of their liquid counterparts. The first of these products includes a mixture of enzymes and inhibitors to enable robust detection of RNA via hydrolysis-probe-based RT-qPCR.

LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001) contains the same versatile features and strong performance as the liquid version: Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019). Performance in multiplexing applications has been optimized, with sensitive, linear detection of up to 5 targets across a range of inputs. The stable cake can be resuspended to make a 2X or 4X mix to accommodate a variety of sample input volumes.

Advantages:
• Simply add nuclease-free water for rapid rehydration
• Store at room temperature for up to 2 years prior to rehydration
• Eliminate cold chain shipping requirements
• Same product performance as liquid format (#M3019)
• Developed in collaboration with Fluorogenics Limited, a wholly owned subsidiary of New England Biolabs, Inc.

To learn more, please visit www.neb.com/L4001

Ordering information:

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<td>L4001S</td>
<td>120 rxns</td>
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<tr>
<td>Nuclease-free Water</td>
<td>B1500S/L</td>
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Lyophilized and liquid Luna RT-qPCR mixes demonstrate equivalent strong performance

RT-qPCR targeting human β-actin was performed using either the LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001) or Luna Probe One-Step 4X Mix with UDG (NEB #M3019) over an 8 log range of input template concentrations (1 μg – 0.1 pg Jurkat total RNA) with 4 replicates at each concentration, run on an ABI QuantStudio 6 Flex real-time instrument. Reactions (20 μl) included primers at 400 nM each and probe at 200 nM, and followed the product recommended cycling conditions.

LYOPHILIZED PRODUCTS FOR MDx OR OEM?

The ability to develop complex, yet simple to use lyophilized products enables us to provide a more complete solution for our customers, particularly those in the molecular diagnostics space.

We have now extensive experience freeze-drying some of NEB’s most popular amplification products, effectively reducing the research and development timelines of custom products.

To learn more about lyophilized reagents from NEB, visit www.neb.com/LyoPrime
LunaScript® Multiplex One-Step RT-PCR Kit – for virtually "error-free" multiplexing PCR

The LunaScript Multiplex One-Step RT-PCR Kit offers a streamlined protocol for cDNA synthesis and extremely high fidelity PCR amplification in a single reaction. The 5X reaction mix contains dNTPs and is optimized for robust multiple target detection in a simple workflow. The 25X enzyme mix features Luna WarmStart Reverse Transcriptase and Q5 Hot Start High-Fidelity DNA Polymerase offering the highest fidelity amplification available making it an ideal choice for next-generation sequencing, library construction DNA arrays, fragment analysis, electrophoresis and traditional cloning/sequencing workflows.

Advantages:

- Closed-tube system with cDNA synthesis and endpoint PCR amplification in a single protocol
- Detect as low as 0.01 pg of human total RNA
- Highest fidelity multiplexing capacity supports use in ARTIC workflows, DNA arrays, cloning & sequencing etc.
- Aptamer-based enzyme control for room temperature setup and stability up to 24 hours

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To learn more, please visit www.neb.com/E1555

WarmStart® Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)

The WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) is fully buffered and compatible with different sample types, enabling multiple detection methods including turbidity detection, real-time fluorescence detection, and end-point visualization such as colorimetric detection via a metal indicator (e.g., hydroxynaphthol blue). For real-time fluorescence detection, the master mix is available as a kit that includes 50X LAMP Fluorescent Dye. The inclusion of dUTP and thermolabile UDG enables carryover contamination prevention.

Advantages:

- Reduce the risk of carryover contamination with thermolabile UDG and dUTP included in the mix
- Set up reactions at room temperature with our unique dual WarmStart formulation
- Optimized performance for real-time fluorescence and endpoint visualization detection methods

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To learn more, please visit www.neb.com/M1708
NEBNext® Ultra™ II reagents & kits: One central workflow for a wide range of applications – now featuring PCR-free workflows

As sequencing technologies continue to improve and applications expand, the need for compatibility with ever-increasing input amounts and sub-optimal sample quality grows. Reliability and high performance are critical, along with faster turnaround, higher throughput, and automation compatibility.

NEBNext’s line of Ultra II DNA library prep reagents has expanded to include PCR-free workflows, powering high performance without the need for amplification. By eliminating the risk of PCR bias, libraries are a clearer reflection of biology.

The NEBNext Ultra II workflow lies at the heart of NEB’s portfolio for next generation sequencing library preparation, with kits and modules for optimal flexibility. You can be assured your DNA libraries will be of the highest quality and yield, even when starting from extremely low input amounts.

The ULTRA II DNA WORKFLOW is available in convenient kit formats or modules:

End Repair/ dA-Tailing | Adaptor Ligation | *Clean Up/ Size Selection | PCR Enrichment | *Clean Up
Module #E7546 | Module #E7595 | *part of kit #E7103 | Module #M0544 | *part of kit #E7103

NEBNext Ultra II DNA Library Prep Kit for Illumina (#E7645)
NEBNext Ultra II DNA Library Kit *with Purification Beads (#E7103)

Your benefits:
- Highest library yields and quality
- Fewer PCR-cycles /PCR-free optional
- Low input amounts

Hands on time: < 15 minutes
Total time: Just ~ 2:30 – 3:00 hrs

Choose the convenient NEBNext Ultra II DNA Library Prep Kit for:
- Whole Genome Seq
- Standard & Low Input Seq
- ChIP-seq, NICE-seq, Cut&Run-Seq
- Exome Capture
- Targeted Sequencing
- FFPE-Material
- cfDNA ...

The ULTRA II DNA WORKFLOW IS ALSO THE CORE OF:

Enzymatic Methyl-Seq (bisulfite-free) | Directional & non-directional RNA-seq | Enzymatic DNA Fragmentation System | Single Cell/ Low Input RNA Library Prep | SARS-CoV-2/ ARTIC Surveillance Sequencing

TOOLS & RESOURCES
- View performance data in our Technical Notes, which can be downloaded at NEBNextUltraII.com
- View the NEBNext Ultra II DNA protocol video for protocol steps, and tips for optimization
- Find hundreds of peer reviewed publications citing use of NEBNext Ultra II DNA on the product pages at NEB.com

NEB is currently the only company, which is offering really "automation friendly" library preparation kits. The volumes of components are calculated to cover for unavoidable deadvolumes, reactant volumes are in range of most automated platforms. The stability of the NEBNext chemistry allows a broad range of automation strategies.

– Dr. Jürgen Zimmermann, Senior Engineer - Automation GeneCore EMBL Heidelberg, Germany
Use the SELECTION CHART below to determine which NEBNext Ultra II products best suit your needs:

<table>
<thead>
<tr>
<th>FEATURES</th>
<th>ULTRA II DNA LIBRARY PREP</th>
<th>ULTRA II FS DNA LIBRARY PREP</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEATURES</td>
<td>Unprecedented performance, enabling lower inputs and fewer PCR cycles</td>
<td>Perform fragmentation, end repair, and dA-tailing with a single enzyme mix</td>
</tr>
<tr>
<td>ULTRA II INPUT AMOUNTS</td>
<td>500 pg – 1 µg of sheared DNA</td>
<td>100 pg – 500 ng of intact DNA</td>
</tr>
<tr>
<td>AVAILABLE WITH OR WITHOUT BEADS?</td>
<td>• With beads: NEB #E7103</td>
<td>• With beads: NEB #E7435</td>
</tr>
<tr>
<td></td>
<td>• Without beads: NEB #E7645</td>
<td>• Without beads: NEB #E7805</td>
</tr>
<tr>
<td>MODULES AVAILABLE?</td>
<td>• Ultra II End Repair/dA-tailing Module (NEB #E7546)</td>
<td>• Ultra II FS DNA Module (NEB #E7810)</td>
</tr>
<tr>
<td></td>
<td>• Ultra II Ligation Module (NEB #E7595)</td>
<td></td>
</tr>
<tr>
<td>ULTRA II PCR-FREE INPUT AMOUNTS</td>
<td>250 ng – 1,000 ng of sheared DNA</td>
<td>50 ng – 500 ng of intact DNA</td>
</tr>
<tr>
<td>Available with or without beads?</td>
<td>• With beads: NEB #E7415</td>
<td>• With beads: NEB #E7435</td>
</tr>
<tr>
<td></td>
<td>• Without beads: NEB #E7410</td>
<td>• Without beads: NEB #E7430</td>
</tr>
<tr>
<td>Modules available?</td>
<td>• Ultra II End Repair/dA-tailing Module (NEB #E7546)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Ultra II Ligation Module (NEB #E7595)</td>
<td>• Ultra II FS DNA Module (NEB #E7810)</td>
</tr>
<tr>
<td>COMPATIBLE WITH FFPE DNA?</td>
<td>Yes – for improved performance, consider the NEBNext FFPE DNA Repair Mix (NEB #M6630)</td>
<td>Yes – for improved performance, consider the NEBNext FFPE DNA Repair Mix (NEB #M6630)</td>
</tr>
<tr>
<td>COMPATIBLE WITH METHYLOME ANALYSIS?</td>
<td>Yes – Methylome analysis is supported; however, we recommend NEBNext EM-seq™ (NEB #E7120)</td>
<td>No – not compatible due to potential for loss of methyl marks</td>
</tr>
<tr>
<td>COMPATIBLE WITH OXFORD NANOPORE TECHNOLOGIES?</td>
<td>Yes. We recommend the NEBNext Companion Module for Oxford Nanopore Technologies® Ligation Sequencing (NEB #E7180).</td>
<td></td>
</tr>
</tbody>
</table>

Note: NEBNext Multiplex Oligos are available separately. Please visit www.neb.com/oligos for options.

Ordering Information:

<table>
<thead>
<tr>
<th>Product</th>
<th>NEB #</th>
<th>SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEBNext Ultra II DNA Library Prep Kit for Illumina</td>
<td>E7645S/L</td>
<td>24/96 reactions</td>
</tr>
<tr>
<td>NEBNext Ultra II DNA Library Prep with Sample Purification Beads</td>
<td>E7103S/L</td>
<td>24/96 reactions</td>
</tr>
<tr>
<td>NEBNext Ultra II FS DNA Library Prep Kit for Illumina</td>
<td>E7805S/L</td>
<td>24/96 reactions</td>
</tr>
<tr>
<td>NEBNext Ultra II FS DNA PCR-free Library Prep with Sample Purification Beads</td>
<td>E7435S/L</td>
<td>24/96 reactions</td>
</tr>
<tr>
<td>NEBNext Ultra II End Repair/dA-Tailing Module</td>
<td>E7546S/L</td>
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</tr>
<tr>
<td>NEBNext Ultra II Ligation Module</td>
<td>E7595S/L</td>
<td>24/96 reactions</td>
</tr>
<tr>
<td>NEW: NEBNext Ultra II DNA PCR-free Library Prep Kit for Illumina</td>
<td>E7410S/L</td>
<td>24/96 reactions</td>
</tr>
<tr>
<td>NEW: NEBNext Ultra II DNA PCR-free Library Prep with Sample Purification Beads</td>
<td>E7415S/L</td>
<td>24/96 reactions</td>
</tr>
<tr>
<td>NEW: NEBNext Ultra II FS DNA PCR-free Library Prep Kit for Illumina</td>
<td>E7430S/L</td>
<td>24/96 reactions</td>
</tr>
<tr>
<td>NEW: NEBNext Ultra II FS DNA PCR-free Library Prep w. Sample Purification Beads</td>
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<td>24/96 reactions</td>
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<tr>
<td>NEBNext Ultra II FS DNA Module</td>
<td>E7810S/L</td>
<td>24/96 reactions</td>
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<tr>
<td>NEBNext FFPE DNA Repair Mix</td>
<td>M6630S/L</td>
<td>24/96 reactions</td>
</tr>
<tr>
<td>NEBNext Enzymatic Methyl-seq Kit</td>
<td>E7120S/L</td>
<td>24/96 reactions</td>
</tr>
<tr>
<td>NEBNext Enzymatic Methyl-seq Conversion Module</td>
<td>E7125S/L</td>
<td>24/96 reactions</td>
</tr>
<tr>
<td>NEBNext Companion Module for Oxford Nanopore Technologies® Ligation Sequencing</td>
<td>E7180S</td>
<td>24 reactions</td>
</tr>
</tbody>
</table>

For more information incl. an overview of all NEBNext Ultra II Kits and modules etc., please visit www.NEBNext.com or contact your local distributor
Do you use DNA ladders in your daily lab work?

We thought so! We’re confident that you will love our Quick-Load DNA ladders, with their sharp bands, ready-to-use format, and competitive pricing. In addition, our Quick-Load Purple ladders cast no UV shadow, so you’ll never miss a band.

UV shadow comparison
The innovative Gel Loading Dye, Purple (6X) (#B7024S) (Lane 1) included in the Quick-Load Purple Ladders does not cast a UV shadow over the underlying bands, unlike conventional Bromophenol-Blue containing gel loading dyes (Lane 2).

For your convenience:
NEB’s Restriction Enzymes also include a free vial of Purple Loading Dye (6x)!

Your local NEB distributor:

*Get special prices on all Quick Load (Blue and Purple) DNA Ladders as well as on NEB competent cells for cloning (page 7). Campaign ends June 30th, 2022. Please ask your local distributor for details.