Fall/Winter 2022

NEB expressions

a scientific update

IN THIS ISSUE

NEW ENGLAND BioLabs®

Monitoring tick and mosquito infections using LAMP: Screening for EEE and beyond

By Joanne Gibson, Ph.D.

You might say LAMP (loop-mediated isothermal amplification) was a niche application before 2020 and it took a pandemic to find its stride. Researchers involved with LAMP development always knew the potential for point-of-care and field applications – and there was a growing interest – but COVID accelerated things when RT-PCR testing infrastructure became strained. Researchers sought alternative diagnostic tools to improve accessibility. This need highlighted the utility of LAMP in settings outside a specialized molecular laboratory and brought it to the forefront. And now, field applications for LAMP for the diagnosis and surveillance of infectious diseases are gaining traction, particularly in low-resource settings.

The evolution of LAMP for diagnostics and surveillance

The Carlow lab at New England Biolabs has used LAMP technology for infectious disease diagnostics and surveillance for almost a decade. They have developed a suite of LAMP tests to detect insect and tick-borne diseases such as Eastern Equine Encephalitis (EEE), anaplasmosis, Lyme Disease and babesiosis, and various Neglected Tropical Diseases (NTDs), such as lymphatic filariasis (elephantiasis) and onchocerciasis (River Blindness).

While LAMP does not replace PCR, the ability to amplify nucleic acids at one temperature, without the need for a thermocycler to rapidly ramp temperatures, is a huge advantage for point-of-care testing for infectious diseases; agricultural diagnostics such as testing crops for infection; and field surveillance programs involving disease vector insects and ticks. Further, colorimetric LAMP gives a visible-to-the-eye read-out, allowing scientists to dispense with specialized fluorescence detection or electrophoresis equipment to obtain results. And the ability to lyophilize LAMP reagents removes the need for refrigeration at the testing site, making it a valuable assay at low-resource locations that have no, or intermittent, electricity supply. These key features reduce the cost and make nucleic acid detection available outside the lab to populations and regions that otherwise have no access to testing.

LAMP uses four core and two additional "loop" primers, producing a short dumbbell structure that forms the basis for exponential amplification. The need to use a thermocycler and high temperatures to denature the DNA strands is replaced by incorporating a strand-displacing DNA polymerase, which extends the primer while displacing the duplex.

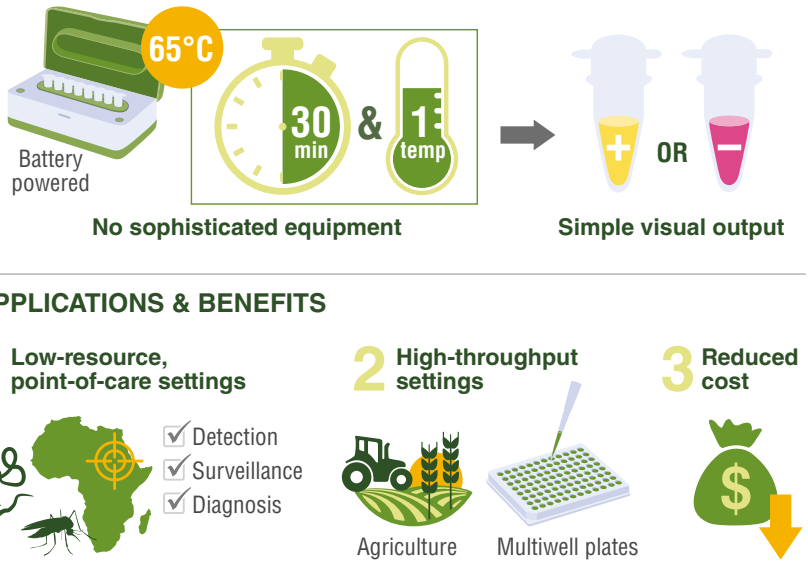
LAMP makes a LOT of DNA, and quickly. A reaction can be complete within 30 minutes. There are various outputs available for the interpretation of results. When the Carlow Lab originally started exploring LAMP, the readout was turbidity-based – it measured the precipitation of magnesium pyrophosphate in the reaction or involved a metal-based colorimetric readout using hydroxynaphthol blue. This wasn't easy to interpret because it involves a change in color from purple to shades of blue. At this point, researchers at NEB developed another colorimetric readout of the LAMP results based on a pH change. Briefly, when the DNA polymerase adds a nucleotide, it produces a pyrophosphate and a proton. Because so much DNA is produced in a LAMP reaction, the number of protons produced is significant, and in a solution that is only moderately buffered, the pH drops by about two units. Including a pH-sensitive dye (phenol red) turns the reaction from pink to yellow, which is much easier to detect by eye.

LAMP is well-suited for diagnostics; however, it doesn't replace PCR for some downstream applications such as cloning. This is because of the structure and length of the product, which can get very long, up to 20,000 – 25,000 bases. The products are concatemers and repeats of the LAMP amplicon sequence with branches, loops, ss/dsDNA, etc. It can be used for sequencing after fragmentation and cleanup if desired, but generally, a rapid yes/no diagnostic answer is sufficient for the intended application.

Fast forward to 2020, and it was quickly realized that LAMP had some application in testing for SARS-CoV-2. Supply chain shortages and labs struggling to keep up with testing needs highlighted the benefit of a diagnostic test that could be applied with minimal cost, materials, and molecular expertise. This brought LAMP to the forefront as many researchers pivoted to address testing needs. NEB scientists established an in-house CLIA-certified SARS-CoV-2 LAMP assay using saliva with minimal sample prep for NEB employees.

At the same time the Carlow Lab was spearheading the CLIA lab LAMP-based testing, they were also involved in various LAMP-based collaborations, namely optimizing a colorimetric Onchocerciasis diagnostic LAMP assay in Cameroon and developing a colorimetric

Figure 1: **The LAMP Workflow is very simple but robust**



The simplified LAMP workflow requires no sophisticated equipment and can be carried out without an electrical source, with results in 30 minutes that are visible to the naked eye. LAMP is ideal for screening and diagnosing NTDs in low-resource settings, agriculture, tick and mosquito monitoring programs, and even at-home testing for infections.

RT-LAMP assay for the detection of Eastern Equine Encephalitis (EEE) virus in vector mosquitoes. Read on to learn more about the latter.

Colorimetric LAMP improves the accessibility of EEEV testing in the U.S.

EEE is an arbovirus transmitted by mosquitoes. Although rare, an infection can cause brain inflammation and is often fatal. It has an enzootic cycle between mosquitoes (*Culiseta melanura* is the primary mosquito vector) and passerine birds. It is seasonal in some areas (such as the U.S. Northeast); however, it is endemic in Florida, providing an ecological niche for sustained wintertime circulation.

While the current gold standard for EEE surveillance is RT-PCR, it is often not implemented because of access and funding limitations. It requires expensive equipment such as a real-time thermocycler. It also requires a level of molecular lab expertise, namely the ability to run RT-PCR tests and interpret threshold (Ct) values and amplification curves. Mosquito control programs around the U.S. typically don't have much funding, and it varies from state to state and even between counties within a state. The cost of sending samples to a specialized lab can stifle a minimally funded surveillance program.

There are 60+ mosquito control programs in Florida that monitor for multiple infections. In addition to EEE, they often monitor West Nile Virus and Saint Louis Encephalitis Virus. Some labs consist only of a cold table (to keep the mosquitoes asleep) and a field microscope (to sort them into different species morphologically). Aerial spraying is initiated once the vector populations reach a threshold level (regardless of whether or not they are infected). Another approach in many counties in Florida relies on EEE infection surveillance in chicken flocks. Samples from the chicken coops are transferred to a central arbovirus lab in Tampa, Florida, where they are assessed for seroconversion. This is a labor-intensive methodology, especially considering the work it takes to maintain chicken coops.

In a study published earlier this year (*Maddison et al., 2022*) in a collaboration between the Carlow lab and Tom Unnasch's lab at the University of South Florida, a LAMP assay was developed to detect EEE virus. It was demonstrated to be a highly accurate alternative to RT-PCR. Professor Unnasch is no stranger to establishing and translating disease surveillance programs into the field; he set up the first routine diagnostic PCR testing lab for River Blindness in Africa in 1992 and works closely with the Florida Mosquito Control Association. The collaboration leveraged Carlow Lab expertise in diagnostics and primer design to produce a tremendously robust assay. The initial primer design process is critical for a successful assay, and NEB scientists and software engineers have streamlined the process with a LAMP Primer Design web tool.

The LAMP assay developed in this collaboration used three highly conserved EEE virus biomarkers (nsP3, E1, and 6K) that target different regions of the viral genome. When RT-PCR was compared with LAMP, 100% agreement was observed between the results from the quantitative (Ct) values obtained with RT-PCR and the end-point color change (pink to yellow). All three biomarkers showed a high level of sensitivity. Of the three biomarkers, E1 and nsP3 were more sensitive than 6K and comparable with the limit of detection using RT-PCR. It should be noted, however, that RT-PCR can detect down to approximately 1/10 of a virus particle, but a single infectious mosquito carries about one million virus particles. So, in that sense, all three RT-LAMP biomarkers were comparable in terms of being able to detect a single infectious mosquito.

Additionally, pooled samples representing homogenates of more than 200 mosquitos, including *Cs. melanura* and also a less competent EEE virus vector, *Culex erraticus*, further demonstrated that RT-LAMP was a highly sensitive alternative for EEE virus detection.

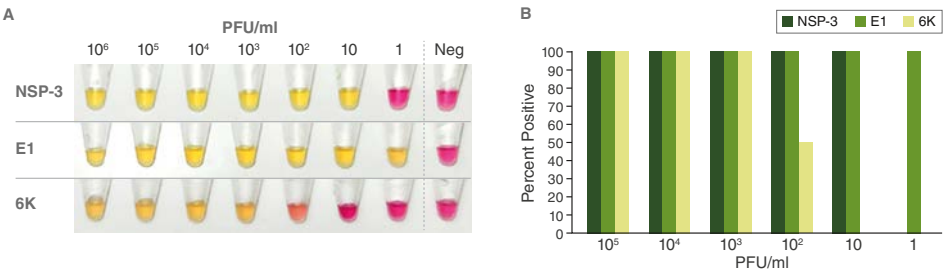
Implementation of LAMP assays in local mosquito programs would require only the addition of a basic hot block (to maintain the amplification temperature at 65°C) to the existing laboratories and very little technical expertise. An added advantage is a reduction in contamination risk because of the simple color change output that can be visualized without opening tubes. The quick turnaround time to run the assay (30 minutes) enables the more extensive screening programs to increase their testing throughput.

All-in-all, this is a cost-effective, simple, fast, and accurate assay that can be implemented at the level of local testing programs; not just for EEE mosquito monitoring, but for many surveillance and diagnostic programs that would benefit from nucleic acid amplification in a simplified format.

This article is taken from the NEBInspired blog.

For references and further information, please visit *neb.com/nebinspired-blog*

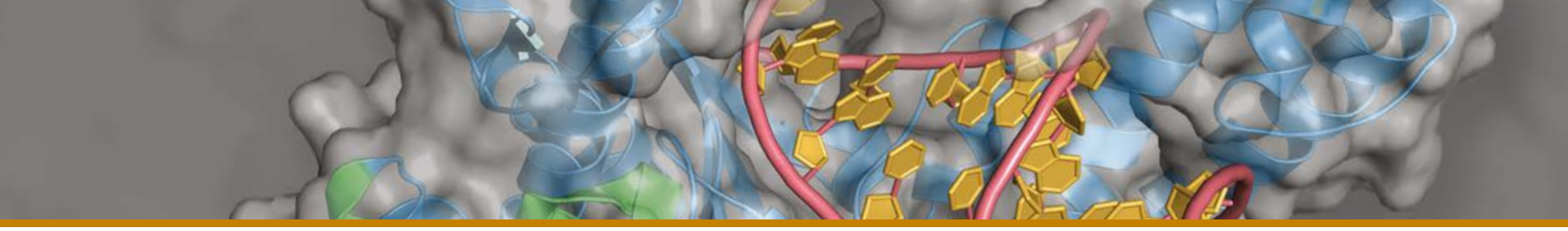
Figure 2: **Analytical sensitivity of RT-LAMP for EEEV.**



(A) Colorimetric RT-LAMP assays targeting nsP3, E1, or 6K, were performed using serially diluted viral RNA (PFU/ml) as a template. A color change from pink to yellow indicates a positive result. (B) The percentage of samples that tested positive at each serial dilution (PFU/ml) of viral RNA using LAMP primers sets for nsP3 (dark green bar), E1 (medium green bar), or 6K (light green bar) are shown (calculation based on biological replicates of each PFU concentration run in duplicate with each primer set).

NEBinspired Blog

NEBinspired is a science blog designed to share inspirational stories about trends in the life sciences, lab tips to help you save time, and life lessons to reflect on.



Fast and reliable DNA or RNA detection by LAMP – Benefit from NEB's superior LAMP Kits!

Loop Mediated Isothermal Amplification (LAMP) is a commonly-used technique for rapid nucleic acid detection (see article page 2-3). NEB's WarmStart LAMP products provide a simple, one-step solution for DNA or RNA targets. The **WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)** (NEB #M1708) 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., hydroxynapthol blue). It features ***Bst* 2.0 WarmStart DNA Polymerase** and **WarmStart RTx Reverse Transcriptase**, both ***in silico***-designed enzymes for improved performance in LAMP reactions. For real-time fluorescence detection, the master mix is available as a kit (NEB #E1708) that includes 50X LAMP Fluorescent Dye.

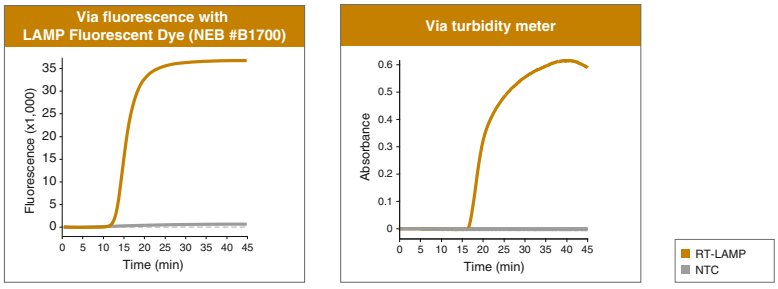
WarmStart Colorimetric LAMP 2X Master Mix with UDG is an optimized formulation of *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx in a special low-buffer reaction solution containing a visible pH indicator for rapid and easy detection of LAMP and RT-LAMP reactions. The inclusion of dUTP and UDG in the master mix reduces the possibility of carryover contamination between reactions.

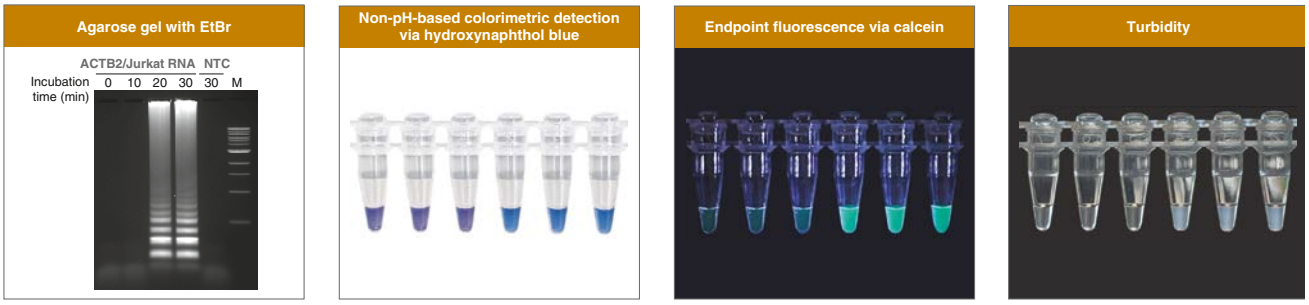
Need help designing LAMP Primers?
Use NEB LAMP Primer Design Tool
lamp.neb.com

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

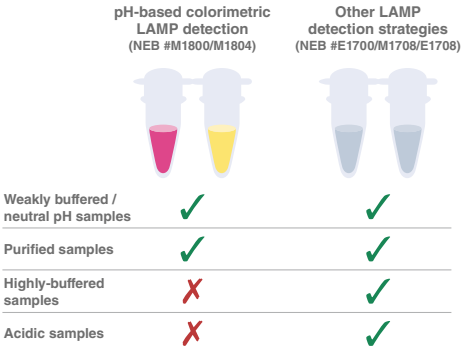
The WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) is compatible with multiple detection methods

A. Real-time detection





LAMP/RT-LAMP master mixes and sample type considerations



NEB's pH-based colorimetric LAMP master mixes with UDG (NEB #M1804) or without UDG (NEB# M1800) are weakly buffered to allow for visual detection of amplification using a pH-sensitive dye. However, the low buffering capacity required to generate the pink to yellow color change limits sample compatibility, as highly buffered sample inputs or acidic samples may impact the change. The WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix with UDG (NEB #M1708, NEB #E1708) or without UDG (NEB #E1700) is fully buffered and can more readily tolerate these types of sample inputs.

Ordering information:

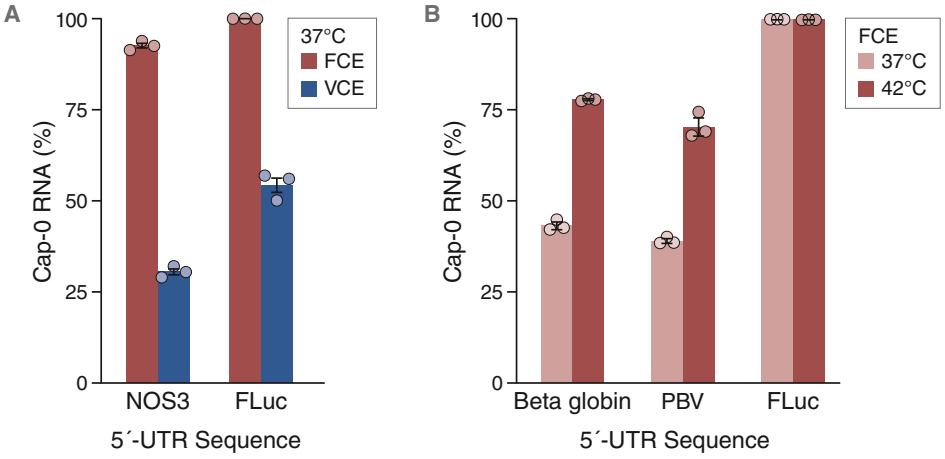
Product	NEB #	Size
WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)	M1708S/L	100/500 reactions
WarmStart Fluorescent LAMP/ RT-LAMP Kit (with UDG)	E1708S/L	100/500 reactions
WarmStart Colorimetric LAMP 2X Master Mix with UDG	M1804S/L	100/500 reactions
Companion Products		
SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit	E2019S	96 reactions

mRNA capping with Faustovirus Capping Enzyme

Looking for a robust, scalable capping solution for your mRNA manufacturing process?

Faustovirus Capping Enzyme (FCE) catalyzes the addition of N7-methylguanosine cap (m7G) to the 5´end of triphosphorylated and diphosphorylated transcripts, producing Cap-0 RNA (1). FCE is a single-subunit enzyme that combines the three activities necessary to produce the Cap-0 structure - triphosphatase, guanylyltransferase, and (guanine-N7)-methyltransferase. FCE retains significant capping activity at low temperatures and tolerates reaction temperatures up to 55°C. In many cases, 1 µl of FCE (25 units) can cap over 100 µg of RNA in 1 hour at 37°C. GTP and S-adenosylmethionine (SAM) are required for capping activity and are included with the enzyme.

FCE offers increased capping efficiency and workflow optimization



A. mRNA capping by FCE and Vaccinia Capping Enzyme (VCE) at 37°C. 200 µg (~350 picomoles, 7 µM) of a 1.77 kb FLuc transcript having 5´-UTR sequences as indicated were treated with a limiting amount of FCE (25 units, 1 picomole, 20 nM in 50µl) or VCE (10 units, 1 picomole, 20 nM in 50 µl) for 1 hour at 37°C. Note that this is less than our recommended amount of enzyme highlighting the increased capping efficiency of FCE vs VCE and the potential benefits of workflow optimization.

B. mRNA capping by FCE at 37°C and 42°C. 200 µg (~350 picomoles, 7 µM) of a 1.77 kb FLuc transcript having 5´-UTR sequences as indicated were treated with a limiting amount (25 units, 1 picomole or 20 nM) of FCE for 1 hour at 37°C or 42°C. Note that this is less than our recommended amount of enzyme highlighting the potential benefits of workflow optimization. All capping reactions were performed in 50 µl reactions containing 0.1 mM SAM, and 0.5 mM GTP, 1X FCE Capping Buffer for FCE reactions or 1X Capping Buffer for VCE reactions. Following capping reactions, mRNA capping was measured using targeted RNase H cleavage and LC-MS.

Reference:
1. Ramanathan, A., et al. (2016) Nucleic Acids Res. 44(16), 7511–7526.

**"GMP-grade" is a branding term NEB uses to describe reagents manufactured under more rigorous infrastructure and process controls to achieve more stringent product specifications and customer requirements. Reagents 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.*

Benefits:

- Experience improved capping efficiency, even on difficult substrates
- Achieve robust capping with less enzyme
- Broad incubation temperature range (37°C – 55°C) for added flexibility
- Choose as an alternative to Vaccinia Capping System, with minimal optimization required
- Enable one-pot Cap-1 synthesis, as FCE is compatible with mRNA Cap 2´-O-Methyltransferase
- Benefit from no licensing fees from NEB for the use of FCE

To learn more, visit
www.neb.com/m2081

Ordering information:

Product	NEB #	Size
Faustovirus Capping Enzyme (FCE)	M2081S/L	500/ 2,500 units
Companion Products		
HiScribe T7 High Yield RNA Synthesis Kit	E2040S	50 reactions
mRNA Cap 2´-O-Methyltransferase	M0366S	2,000 units

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

The finest in PCR fidelity – for over 10 years

How time flies! It has been over 10 years since the release of Q5 High-Fidelity DNA Polymerase. In that time, it has set the standard for PCR performance and fidelity (>280 times higher fidelity than *Taq*). Its unique buffer system provides superior performance for a broad range of amplicons, regardless of GC content.

Q5 is featured in multiple products to support a range of applications, and we are proud to announce our newest release for direct sample amplification: Q5 Blood Direct 2X Master Mix.

Trust Q5 DNA Polymerase

for all your high-fidelity PCR needs

PCR

Q5 High-Fidelity DNA Polymerase
(NEB #M0491)

Q5 Hot Start High-Fidelity DNA Polymerase
(NEB #M0493)

Q5U Hot Start High-Fidelity DNA Polymerase
(NEB #M0515)

Q5 High-Fidelity 2X Master Mix
(NEB #M0492)

Q5 Hot Start High-Fidelity 2X Master Mix
(NEB #M0494)

Q5 High-Fidelity PCR Kit
(NEB #E0555)

NGS LIBRARY AMPLIFICATION

NEBNext Ultra™ II Q5 Master Mix
(NEB #M0544)

NEBNext High-Fidelity 2X PCR Master Mix
(NEB #M0541)

NEBNext Q5 Hot Start HiFi PCR Master Mix
(NEB #M0543)

NEBNext Q5U Master Mix
(NEB #M0597)

ARTIC VIRAL SEQUENCING

One-Step

LunaScript Multiplex One-Step RT-PCR Kit
(NEB #E1555)

Two-Step

LunaScript RT SuperMix
(NEB #M3010)

Q5 Hot Start High-Fidelity 2X Master Mix
(NEB #M0494)

DIRECT SAMPLE AMPLIFICATION

Q5 Blood Direct 2X Master Mix
(NEB #M0500)

MUTAGENESIS

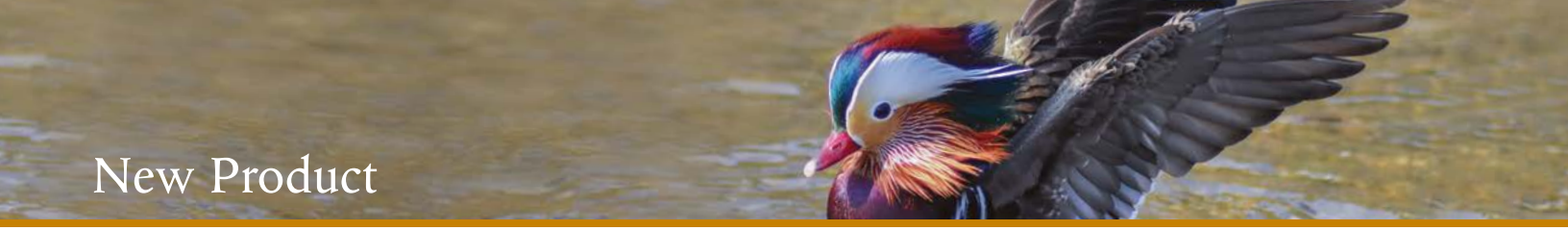
Q5 Site-Directed Mutagenesis Kit
(NEB #E0554)

Q5 Site-Directed Mutagenesis Kit (Without Competent Cells)
(NEB #E0552)

View the full product portfolio and request your free sample at
Q5PCR.com
or contact your local distributor.

Free Sample

Limited Offer:
Ask your local distributor for a free sample of Q5 DNA Polymerase!

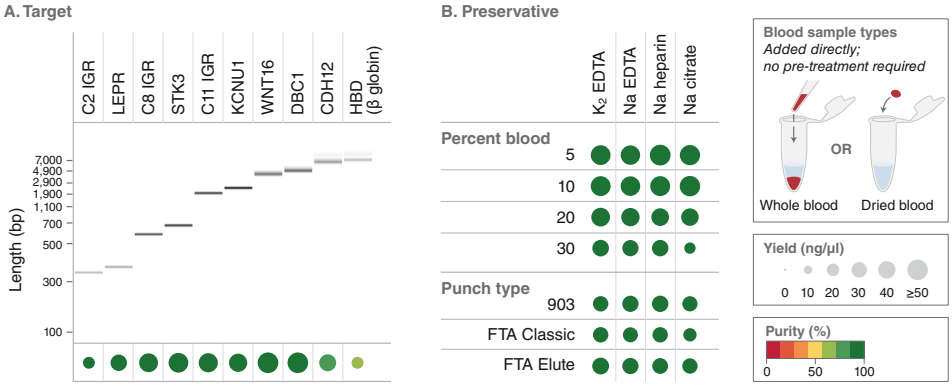


New Product

PCR direct from blood with Q5 Blood Direct 2X Master Mix

Our Q5 portfolio has just expanded to include Q5 Blood Direct 2X Master Mix. This mix can amplify a wide variety of targets directly from dried blood spots or up to 30% whole human blood, skipping DNA purification. The master mix includes Q5 Hot Start High-Fidelity DNA Polymerase and dNTPs in an optimized buffer that delivers increased resistance to inhibitors in blood, anti-coagulants, and chemicals on filter papers. It is capable of amplifying products up to 7.5 kb from human whole blood cells preserved with sodium EDTA, potassium EDTA, sodium citrate and sodium heparin, as well samples stored on common preservative filter papers.

Q5 Blood Direct 2X Master Mix enables robust amplification directly from whole or dried blood



PCR was performed using Q5 Blood Direct 2X Master Mix under standard recommended conditions with 35 cycles of amplification. Yield and purity were quantitated by microfluidic LabChip analysis and are indicated by dot size and color, respectively, with a large, dark green dot representing the strongest performance.

A. Amplification of a variety of human genomic amplicons, 0.3 to 7.5 kb in length, from 10% EDTA-preserved human whole blood. Results are shown as both a virtual gel (top) and corresponding dot plot (bottom). Q5 Blood Direct 2X Master Mix performs well across a broad range of amplicon sizes.

B. Amplification of a 604 bp human genomic amplicon from whole blood (top) or blood dried on filters (bottom). Human whole blood comprised 5-30% of the total reaction volume (50 µl) as indicated. Untreated 1 mm punches from dried blood spots were added directly to 25 µl reactions (one punch per reaction), even where pre-treatment of the punch was recommended by the manufacturer. Q5 Blood Direct 2X Master Mix shows broad tolerance to varying blood volumes, preservatives and punch types.

To request a sample, visit
www.neb.com/m0500
or contact your local distributor.

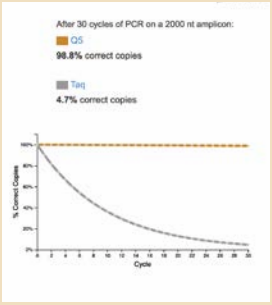
Benefits:

- Skip DNA purification and amplify direct from human whole blood cells or dried blood spots
- Utilize Q5 Hot Start High-Fidelity DNA Polymerase for accurate and efficient PCR
- Benefit from increased resistance to inhibitors and chemicals with our optimized buffer system
- Set up reactions at room temperature with aptamer-based enzyme control

Limited Offer:
Ask your local distributor for a free sample of Q5 Blood Direct 2x Master Mix!

Ordering information:

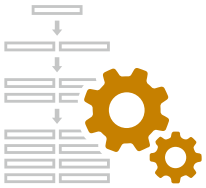
Product	NEB #	Size
Q5 Blood Direct 2X Master Mix	M0500S/L	100/500 reactions



PCR Fidelity Estimator!

Estimate the percentage of correct DNA copies (those without base substitution errors) per cycle of PCR for selected DNA polymerases and see why Q5 DNA Polymerase is your preferred high-fidelity polymerase.

Try out the free PCR Fidelity Estimator
PCRFidelityEstimator.neb.com



General Guidelines for **PCR Optimization**

New England Biolabs offers a diverse group of DNA Polymerases for PCR-based applications. Specific recommendations for PCR optimization can be found in the product literature or on the individual product webpages. However, these general guidelines will help to ensure success using our PCR enzymes.

Setup Guidelines

DNA Template

- Use high quality, purified DNA templates whenever possible. Please refer to specific product information for amplification from unpurified DNA (e.g., colony PCR or direct PCR).
- For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg-10 ng of DNA per 50 µl reaction
- For higher complexity templates (e.g., genomic DNA), use 1 ng-1 µg of DNA per 50 µl reaction
- Higher DNA concentrations tend to decrease amplicon specificity, particularly for high numbers of cycles

Primers

- Primers should typically be 20-30 nucleotides in length, with 40-60% GC Content
- Primer Tm values should be determined with NEB's Tm Calculator (TmCalculator.neb.com)
- Primer pairs should have Tm values that are within 5°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between the primers
- Higher than recommended primer concentrations may decrease specificity
- When engineering restriction sites onto the end of primers, 6 nucleotides should be added 5´ to the site
- Annealing temperatures should be determined according to specific enzyme recommendations. ***Please note that Q5 and Phusion* annealing temperature recommendations are unique***
- Final concentration of each primer should be 0.05-1 µM in the reaction. Please refer to the more detailed recommendations for each specific enzyme
- When amplifying products > 20 kb in size, primers should be ≥ 24 nucleotides in length with a GC content above 50% and matched Tm values above 60°C
- To help eliminate primer degradation and subsequent non-specific product formation, use a hot-start enzyme (e.g., One***Taq*** Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)

**Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion is a registered trademark and property of Thermo Fisher Scientific.*

Magnesium Concentration

- Optimal Mg^{2+} concentration is usually 1.5-2.0 mM for most PCR polymerases
- Most PCR buffers provided by NEB already contain sufficient levels of Mg^{2+} at 1X concentrations
- NEB offers a variety of Mg-free reaction buffers to which supplemental Mg^{2+} can be added for applications that require complete control over Mg^{2+} concentration
- Further optimization of Mg^{2+} concentration can be done in 0.2-1 mM increments, if necessary. For some specific applications, the enzyme may require as much as 6 mM Mg^{2+} in the reaction
- Excess Mg^{2+} may lead to spurious amplification; Insufficient Mg^{2+} concentrations may cause reaction failure

Deoxynucleotides

- Ideal dNTP concentration is typically 200 µM of each, however, some enzymes may require as much as 400 µM each. Please refer to specific product literature for more detailed recommendations
- Excess dNTPs can chelate Mg^{2+} and inhibit the polymerase
- Lower dNTP concentration can increase fidelity, however, yield is often reduced
- The presence of uracil in the primer, template, or deoxynucleotide mix will cause reaction failure when using archaeal PCR polymerases. Use One***Taq*** or ***Taq*** DNA Polymerases for these applications

Enzyme Concentration

- Optimal enzyme concentration in the reaction is specific to each polymerase. Please see the product literature for specific recommendations
- In general, excess enzyme can lead to amplification failure, particularly when amplifying longer fragments

Starting Reactions

- Unless using a hot start enzyme (e.g., One***Taq*** Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase), assemble all reaction components on ice
- Add the polymerase last, whenever possible
- Transfer reactions to a thermocycler that has been pre-heated to the denaturation temperature. Please note that pre-heating the thermocycler is not necessary when using a hot start enzyme (e.g., One***Taq*** Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)

Cycling Guidelines

Denaturation

- Optimal denaturation temperature ranges from 94°C-98°C and is specific to the polymerase in the reaction. Please refer to product information for recommended conditions
- Avoid longer or higher temperature incubations unless required due to high GC content of the template
- For most PCR polymerases, denaturation of 5-30 seconds is recommended during cycling
- NEB's aptamer-based hot start enzymes do not require additional denaturation steps to activate the enzymes

Annealing

- Primer Tm values should be determined using the NEB Tm Calculator (TmCalculator.neb.com)
- For PCR polymerases other than Q5 High-Fidelity DNA Polymerase or Phusion High-Fidelity DNA Polymerase*, annealing temperatures are usually set at 2°C-5°C below the lowest Tm of the primer pair
- When using Q5 High-Fidelity DNA Polymerase or Phusion High-Fidelity DNA Polymerase*, annealing temperatures should be set at 0°C-3°C above the lowest Tm of the primer pair. Please refer to the product literature for detailed recommendations
- Non-specific product formation can often be avoided by optimizing the annealing temperature or by switching to a hot start enzyme (e.g., One***Taq*** Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)
- Annealing temperatures can be optimized by doing a temperature gradient PCR, starting at 5°C below the lowest Tm of the primer pair
- Ideally, primer Tm values should be less than the extension temperature. However, if Tm values are calculated to be greater than the extension temperature, a two-step PCR program (combining annealing and extension into one step) can be employed

Extension

- Extension temperature recommendations range from 65°C-72°C and are specific to each PCR polymerase. Please refer to the product literature for specific recommendations
- Extension rates are specific to each PCR polymerase. In general, extension rates range from 15-60 seconds per kb. Please refer to the recommendations for each specific product
- Longer than recommended extension times can result in higher error rates, spurious banding patterns and/or reduction of amplicon yields

Monarch DNA & RNA Purification Kits – Designed with sustainability in mind

At NEB, we continuously strive to promote ecologically sound practices and environmental sustainability in order to protect our natural resources, both locally and globally. For over 5 years, we have been designing our Monarch DNA & RNA purification kits & products with sustainability in mind by purposefully reducing plastics and packaging without sacrificing performance & quality.

Reduced lab waste

Significantly less plastic as compared to leading supplier
Monarch kits still deliver high yields, purity and performance

Thinner-walled columns
Reduction in total plastic without affecting performance

Buffer bottles
Carefully designed to minimize plastic usage

Flexible purchasing options

Buffers and columns sold separately
Purchase only what you need and avoid wasted materials

Same performance, design and formulations
Standalone products are the same components that are included in complete kits

MONARCH®
Sustainability

No excessive packaging

Sturdy, reusable boxes at just the right size
Carefully designed to eliminate empty space, versatile Monarch boxes can be reused anywhere

Concise protocol cards replace printed manuals
Both cards and manuals are available online as PDFs

Sustainable & recyclable packaging

Sourced for recyclability
All components are purposefully sourced for recyclability

Instructions for recycling kit components
Can be found on product packing or online

Recycled paper
Used to make the kit boxes, inserts and paper materials

Eco-friendly printing
Printing of boxes and packaging powered by green sustainable sources such as wind

We hope that others in the scientific community join us in our efforts to make nucleic acid purification greener, one step at a time.

To learn more and request your Monarch sample, visit
NEBMonarch.com

The core of your NGS Library Prep

NEBNext Ultra II reagents & kits: One central workflow for a wide range of applications

Spot on: NEBNext Ultra II RNA-Seq

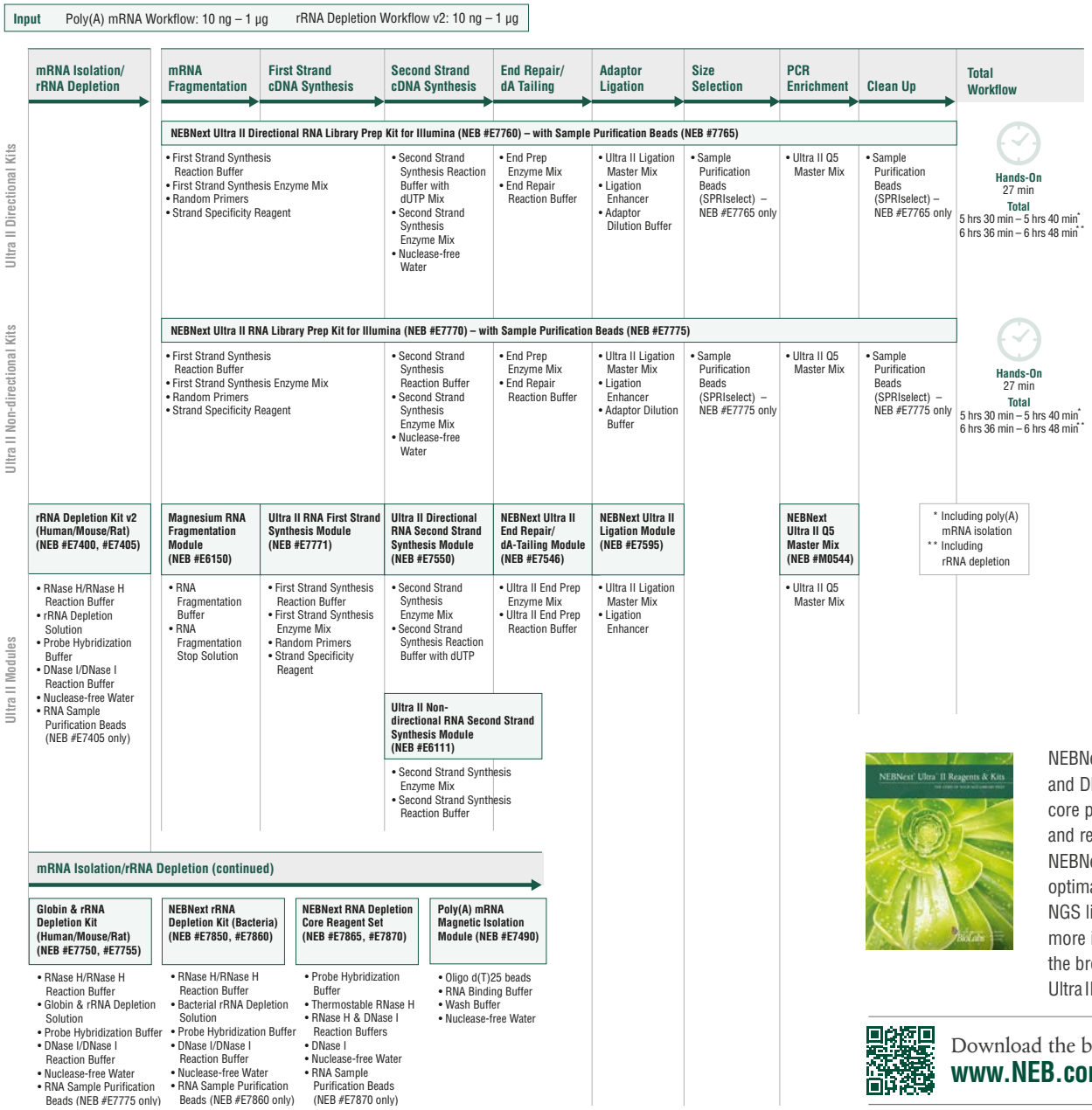
RNA-seq is commonly used to determine the presence and quantity of RNA species in a sample enabling sensitive and accurate gene expression analysis. NEBNext Ultra II RNA kits enable you to build excellent NGS libraries from even low input amounts. Whether you prefer directed (i.e. strand-specific) or undirected RNA sequencing, NEBNext Ultra II RNA kits offer you fastest workflows with minimal hands-on time.

NEBNext Ultra II RNA kits are your preferred choice at any scale as they are already automated on various leading liquid handling platforms. Contact your local distributor for more information.

Free Sample

Limited Offer:
Ask your local distributor for a free sample of your favorite NEBNext kit!

NEBNext kit components



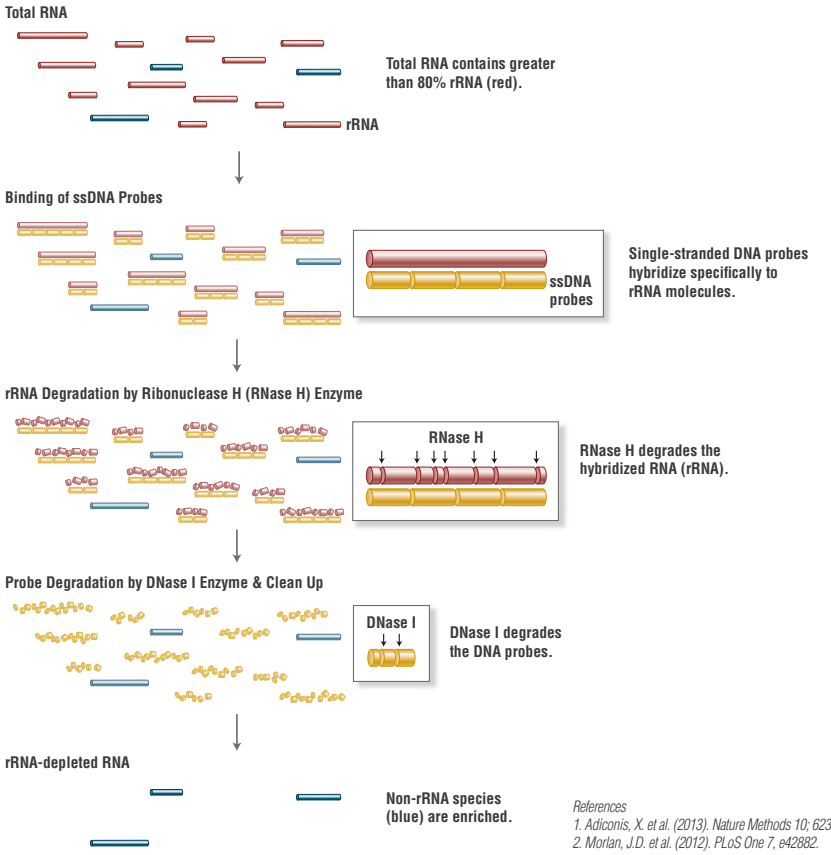
NEBNext Ultra II RNA and DNA kits share core protocol steps and reagents making NEBNext Ultra II the optimal solution for any NGS library prep. Find more information in the brochure "NEBNext Ultra II DNA & RNA Kits".

Download the brochure at
www.NEB.com/literature

NEBNext RNA Depletion

Abundant RNAs can conceal the biological significance of less abundant transcripts, and so their efficient and specific removal is desirable. NEBNext RNA Depletion kits facilitate this removal, while ensuring retention of RNAs of interest. These kits employ the efficient RNase H method (1,2), as well as close probe tiling of abundant RNAs, thereby ensuring that even degraded RNA is hybridized and subsequently removed.

NEBNext rRNA Depletion Kit workflow



References
1. Adiconis, X. et al. (2013). Nature Methods 10; 623-629.
2. Morlan, J.D. et al. (2012). PLoS One 7, e42882.

Total RNA (0.1-1 µg) is hybridized with single stranded DNA probes targeting cytoplasmic (5S, 18S, 28S, 5.8S rRNAs) and mitochondrial (12S and 16S rRNAs) ribosomal RNA, followed by RNase H digestion to degrade targeted RNA. Finally, DNA probes are digested with DNase I. The ribosomal-depleted RNA is purified using Agencourt RNAClean XP beads. Ribosomal RNA depletion can be immediately followed by RNA-seq library preparation.

NEBNext rRNA Depletion Kit workflow times

	Input Amount	Time				Workflow Time
		RNA/Probe Hybridization	RNase H Digestion	DNase I Digestion	Clean Up	
Kit	10 ng – 1 µg	Hands-On				Hands-On
		2 min.	2 min.	2 min.	2 min.	8 min.
		Total				Total
		22 min.	32 min.	32 min.	27 min.	1 hr., 53 min.

- Suitable for low-quality (e.g., FFPE) and high-quality RNA
- Compatible with a broad range of input amounts: 10 ng-1 µg
- Superior depletion of abundant RNAs, with retention of RNAs of interest
- Fast workflow: 2 hours, with less than 10 minutes hands-on time
- Depleted RNA is suitable for RNA-seq, random-primed cDNA synthesis, or other downstream RNA analysis applications

Customized depletion of unwanted RNAs of any species:

- **STEP 1:** Use the online ***NEBNext Custom RNA Depletion Design Tool*** to obtain custom probe sequences, by entering the sequence of your target RNA
- **STEP 2:** Order ssDNA probe oligonucleotides from your trusted oligo provider
- **STEP 3:** Use the probes with the ***NEBNext Custom RNA Depletion Core Reagent Set (NEB #E7865)*** or in combination with other NEBNext RNA Depletion Kits

Ordering information:

Product	NEB #	Size
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)	E7400S/ L/X	6/24/96 reactions
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads	E7405S/ L/X	6/24/96 reactions
NEBNext Globin & RNA Depletion Kit	E7750S/ L/X	6/24/96 reactions
NEBNext rRNA Depletion Kit (Bacteria)	E7850S/ L/X	6/24/96 reactions
NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads	E7860S/ L/X	6/24/96 reactions
Tip: NEBNext RNA Depletion Core Reagent Set – ***for "any" species***	E7865S/ L/X	6/24/96 reactions
Also Available		
NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 reactions

Attention lecturers, course instructors, professors,...

Are you involved in student education or training?

NEB supports you with the free ***NEB STEM Education Pack*** for basic scientific education of future molecular biologists!

Take advantage of our special ***"STEM Education Discount"*** on individual NEB reagents and kits for your class! Moreover, we assist you with free sets of topic-specific NEB literature for all your students e.g.:

- The NEB Catalog and Technical Reference 2021/22 with 80+ pages tips and tricks and references
- The Molecular Cloning Technical Guide...

We also support you with ***free tools for your lab bench,*** e.g.:

- NEB tube rack
- NEBCool lab bench cooler
- NEB Monarch water bath floatie
- NEBTimer
- NEB pull-reel key ring
- NEB highlighter

We tailor the content of the limited *NEB STEM Education Pack* to meet your needs. Please contact your local distributor to discuss how we can support you best!

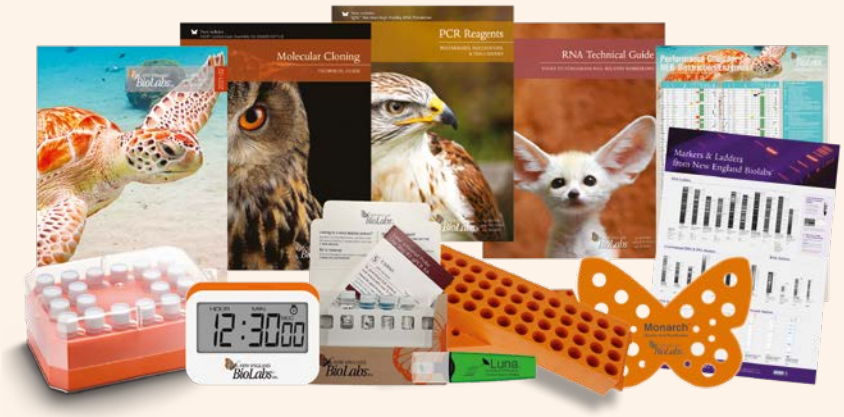


Image for printing purpose only. Content of individual NEB STEM Education Pack varies.
Limited offer closing December 31st, 2022 or as long as stocks last.
Please check availablbility with your local distributor.

Your local NEB distributor:

BeNeLux:
BIOKÉ
Tel: (+31) 71 720 0220
(BE: 0800 - 71640)
info@bioke.com
www.bioke.com

BULGARIA
ELTA 90M Ltd.
Tel: (+359) 2 983 96 49
Fax: (+359) 2983 2211, 9274 232
office@elta90.eu
www.elta90.com

CROATIA:
Diagnostica Skalpeli d.o.o.
Tel: (+385) 1 377 8484
Fax: (+385) 1 377 8585
skalpeli@skalpeli.hr
www.skalpeli.hr

CZECH REPUBLIC:
BioTech a.s.
Tel: (+420) 2727 017 39
info@ibiotech.cz
www.ibiotech.cz

DENMARK:
BioNordika Denmark A/S
Tel: (+45) 39 56 20 00
Fax: (+45) 39 56 19 42
info@bionordika.dk
www.bionordika.dk

FINLAND:
BioNordika Oy
Tel: (+358) 207 410 270
info@bionordika.fi
www.bionordika.fi

GEORGIA
Oxgen-Import, LLC
Tel: (+99) 559 937 43 74
Email: info@oxgenimport.ge
Website: www.oxgen.ge

GREECE:
BIOLINE SCIENTIFIC
Tel: (+30) 210 522 6547
Fax: (+30) 210 524 4744
info@bioline.gr
www.bioline.gr

HUNGARY:
Kvalitex Kft.
Tel: (+361) 340 4700
Fax: (+361) 339 8274
info@kvalitex.hu
www.kvalitex.hu

ITALY:
Euroclone S.p.A.
Tel: (+39) 02 38 1951
info@euroclone.it
www.euroclonegroup.it

ISRAEL:
Ornat BioChemicals &
Lab Equipment
Tel: (+972) 8 947 7077
Fax: (+972) 8 936 3034
ornatbio@ornat.co.il
www.ornat.co.il

KAZAKHSTAN
NOS, LLC
Tel: (007) 7172 967 543
Email: market@inos.kz
Website: inos.kz

LITHUANIA:
Nanodiagnostika, Ltd.
Tel (+370) 525 052 44
lina@nanodiagnostika.lt
www.nanodiagnostika.lt

NORWAY:
BioNordika AS
Tel (+47) 2303 5800
info@bionordika.no
www.bionordika.no

POLAND:
Lab-JOT Ltd. Sp.z o.o. Sp.k.
Tel: (+48) 2233 598 84
Fax: (+48) 2233 598 19
biuro@labjot.com
www.labjot.com

PORTUGAL:
Werfen
Tel. (+351) 214 424 7300
dac-pt@werfen.com
www.pt.werfen.com

ROMANIA:
BioZyme
Tel: (+40) 264 523 281
contact@biozyme.ro
www.biozyme.ro

SERBIA:
Alfa Genetics d.o.o.
Tel: (+38) 1011 2084 252
office@alfagenetics.rs
www.alfagenetics.rs

SPAIN:
Werfen
Tel: (+34) 934 010 101
customerservice-es@werfen.com
www.es.werfen.com

SLOVAK REPUBLIC
BioTech s.r.o.
Tel: (+421) 254 7744 88
Fax: (+421) 232 2010 48
info@ibiotech.sk
www.ibiotech.sk

SLOVENIA:
Mikro+Polo d.o.o.
Tel: (+386) 2614 33 00
Fax: (+386) 2614 33 20
info@mikro-polo.si
www.mikro-polo.si

SOUTH AFRICA:
Inqaba Biotechnical Industries
(Pty) Ltd
Tel: (+27) 1234 358 29
info@inqababiotec.co.za
www.inqababiotec.co.za

SWEDEN, ESTONIA, LATVIA,
LITHUANIA:
BioNordika Sweden AB
Tel: (+46) 830 60 10
info@bionordika.se
www.bionordika.se

SWITZERLAND:
BioConcept Ltd.
Tel: (+41) 6148 680 80
Fax: (+41) 6148 680 00
info@bioconcept.ch
www.bioconcept.ch

TURKEY:
EKA BIOLAB TEKNOLOJI
Tel. (+90) 232 570 37 83
Fax: (+90) 232 570 37 02
Email: info@ekabiolab.com
www.ekabiolab.com

For a complete list of NEB subsidiaries and distributors, please go to:
www.neb.com/international-support

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices
Certified B Corporation

www.neb.com

NEW ENGLAND BioLabs®

be INSPIRED
drive DISCOVERY
stay GENUINE