

Key considerations for optimal lyophilized reagent development

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A wholly-owned subsidiary of New England Biolabs[®] (NEB[®]), New England Biolabs Lyophilization Sciences works in concert with NEB to provide lyophilized nucleic acid amplification reagents to the research, applied and diagnostics markets. In May 2021, NEB acquired Fluorogenics Limited[™] (FGL), facilitating the combination of NEB's expertise in enzymology with FGL's extensive understanding of the lyophilization process. This innovation, expertise and know-how enables us to deliver a best-in-class solution for your lyophilization needs. The purpose of this paper is to explain the lyophilization development process, as well as costs, capabilities, and supply options associated with customized lyophilized projects, in order to streamline your product development.

WHEN IS LYOPHILIZATION RECOMMENDED?

Although frozen reagents are an acceptable and reasonable solution for most laboratory applications, they require a robust cold chain and may be susceptible to impaired performance due to improper transport and storage. The effect of temperature changes and defrosting is dependent on the functional specification of the chosen frozen product; reverse transcriptases, for example, are more labile than thermostable DNA-dependent DNA polymerase enzymes. When designed well, lyophilized products are far more robust to transient changes in temperature and offer an ideal solution to low-cost transport as they do not require a cold chain or special handling at ports of entry or upon receipt. For applications that benefit from reagent robustness over long time periods, such as extended trials or diagnostics, lyophilized products can provide greater uniformity, will offer a longer shelf-life and can be customized for numerous workflows.

LYOPHILIZATION REQUIREMENTS

The lyophilization process requires different protectants and stabilizers to those used in frozen or liquid mixes. Indeed, many cryo-protectants used in frozen formulations (e.g., glycerol) are inhibitory to the freeze-drying processes used to create a dry master mix product. For this reason, it is not typically possible to freeze-dry most liquid master mixes directly. However, most lyo providers work with commercially available reagents and process enzymes and other raw materials from external suppliers without the benefit of compositional details or sufficient support. This is feasible, but unnecessarily challenging.

LYOPHILIZATION OF CUSTOM PRODUCTS

The combination of enzyme expertise and lyophilization capabilities enables NEB Lyo Sciences to create lyophilized products that do not sacrifice the high-performance qualities of their liquid counterparts. NEB provides core lyophilized formulations through its catalog business which can be used for a variety of purposes, including early-stage testing in the research and molecular diagnostics space, lowering the barrier to initial proof-of-principle studies or iterative development trials.

Where customization is needed, NEB Lyo Sciences can also build upon these core reagents to include primers, probes and other solutes to make your lyophilized product a 'ready-to-use' solution. This greatly simplifies testing and removes the need for automation while also eliminating human and operational errors. By building upon reagents that have already been optimized and lyophilized, the process of creating a custom formulation is simplified, reducing the time it will take to create modified versions.

When moving from the catalog offering to a custom product, members of our technical and project management teams will meet with you to understand your project, goals, and timelines as well as your detailed requirements. Information collected during this discovery phase will include details about your intended application (target market, desired price points, end user workflow), reagent requirements (necessary enzyme properties, buffers, salts, other solutes), assay design (reagent volumes, sample inputs, primers/probes), and desired packaging formats (glass vials, PCR tubes, plates, custom

cartridges). Providing as much detail as possible early in the discovery phase will enable us to design and produce a product that will meet your performance and manufacturing needs. In areas of design flexibility, we will offer assistance to ensure compatibility with scale-up and manufacturing.

RAW MATERIALS FOR CUSTOM PRODUCTS

Our team can provide a variety of materials and biologics to meet functional and cost requirements for each custom project. We can manage the supply chain for your enzymes and other components as needed. Custom products may draw on the NEB portfolio of enzymes and biologics and the combined expertise across our organizations to provide your specification lyophilized from a single provider.



Environmentally Friendly Global Distribution: Lyophilized products are not constrained by supply logistics.

THE LYOPHILIZATION PROCESS

Lyophilization, or freeze-drying, involves freezing a reagent mixture containing cryo-protectants (also known as excipients) at low temperatures (~ -40°C) and pressures (~100 mTorr) such that the water within the mixture sublimates from the solid to vapor phase. Excipients typically replace water and are chosen to protect biologics such as enzymes.

Product lyophilization at NEB Lyo Sciences is carried out in a freeze dryer – a dedicated instrument that automates the drying process. Freeze dryers are batch-based devices, typically containing multiple shelves. Dryer capacity is defined by the number of shelves and the density of the material that can be added per shelf. The primary packaging of a reagent (vial, plate, cartridge, etc.) must maintain contact with the shelf to ensure a good thermal contact to facilitate drying, therefore the shape and form of the packaging should be considered when designing a workflow, container or consumable device. For custom containers, we can design custom tooling to enable shelf contact and achieve an optimally dried product. The freeze-drying development process includes carefully optimized step-changes in both temperature and pressure to thoroughly dry a product while ensuring that full reagent activity can be maintained upon rehydration. This process may vary with reagent formulations, so research and development is often needed to optimize the drying of new enzymes/components.

NEB Lyo Sciences has extensive experience freeze drying some of NEB's most popular amplification products, effectively reducing the research and development timelines of custom products based on these reagents. State-of-the-art analytical tools and processes allow us to quickly optimize process parameters. In addition, NEB's use of engineered enzymes with enhanced

stability and performance plus aptamers that control room temperature enzyme activity simplifies the lyophilization process, extending the stability of formulations through the key step of product dispensing prior to lyophilization.

THE DRY FORMULATION

When the right excipients are used, the active materials in a product (e.g., enzymes) will be protected at ambient temperatures in the same way that they are in a frozen product. When lyophilization is done well, there is no need to increase the amount of active ingredients (e.g., enzymes) in a dried product to achieve a desired activity. Spending time to get a formulation correct early can bring significant long-term savings over the life of a project/product.

Over the years, NEB Lyo Sciences has evaluated numerous excipients and developed an in-depth understanding of their benefits and limitations. Additionally, NEB Lyo Sciences has proprietary excipient formulations that enable excellent protection of biologics while also permitting instant resuspension. The most popular excipients exhibit desiccating properties such that instant dissolution of the product is maintained even after exposure to high humidity environments. This is an invaluable feature when using lyophilized products in automated workflows and in integrated systems. NEB Lyo Sciences can also offer an excipient formulation that will enable your product to adhere to plasticware, if desired.

PRIMARY PACKAGING & PRODUCT FORMAT

As noted previously, the amount of material that can be dried in a single freeze dryer is dependent upon the specifications of the dryer, but also by the primary packaging footprint. Product height plays a lesser role, provided it does not impact the



LyoPrime Luna™ Probe One-Step RT-qPCR Mix with UDG (NEB #L4001)

number of dryer shelves that can be used. Packaging with a higher volume-to-shelf surface area will produce more product per unit shelf area and will therefore be less expensive to develop and manufacture.

NEB Lyo Sciences manufactures nucleic acid amplification-based products in crimped glass vials, screw capped vials, 96-well plates, PCR strips and in a variety of integrated platform consumables. Containers that can be closed within the dryer, such as butyl-stoppered glass vials with crimped or screw caps, are an ideal choice for lyophilized reagents. These glass vials are highly resistant to both moisture and oxygen ingress, allowing for an extended product shelf life at room temperature. Glass vials are also an ideal size for running high-density dryer batches, and thus offer the most straightforward and cost-effective product formatting.

Plastic containers such as PCR plates are made from polypropylene, which are pervious to both moisture and oxygen. For most projects, PCR plates and strips require secondary packaging to provide a seal that protects against moisture and oxygen – for example metalized foil pouches. Other vials that do not close within the dryer may also require secondary packaging to exclude moisture and oxygen. NEB Lyo Sciences has custom packaging cabinets to apply secondary packaging in an inert atmosphere, providing longevity for your products.

QC & QUALITY ASSURANCE

Depending upon your product needs, NEB Lyo Sciences can carry out functional and materials testing. Functional testing of custom products verifies post-drying performance. NEB Lyo Sciences has thermocyclers and lab automation tools to qualify your products as soon as drying is completed. Materials testing may include residual moisture determination using Karl Fischer analysis. This provides important infor-

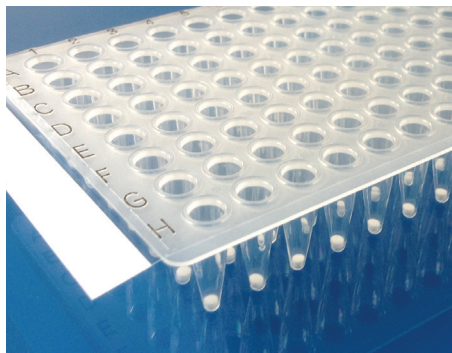


A typical freeze dryer

mation about the success of the research and development phase and provides an important measurement for high value product QC and release.

During the research and development of your products, NEB Lyo Sciences takes a stepwise approach, first formulating and lyophilizing in vials (if not already complete), then perfecting formulation and confirming dryness. Analytical development tools such as freeze-drying microscopy can identify key drying process parameters. In-process analysis such as “pressure rise” and “transition” analysis assist with process optimization and scale-up. This ensures both concrete science and value for your development program with reduced risks at each stage.

NEB Lyo Sciences operates under an ISO 13485:2016 compliant quality management system with a certified scope covering contract design, development, and manufacture of molecular diagnostic reagents for *in vitro* diagnostic medical devices. This provides the assurance for manufacturers of IVD products that our products are positioned to deliver products that meet all necessary requirements. Quality audits are routinely performed by our customers or other external agencies, as required.



96-well plate format



Glass vials are ideal for reagent manufacture: glass is impervious to both oxygen and moisture

DEVELOPMENT & MANUFACTURING COSTS

Understanding your specific project requirements, timelines and predicted volumes will enable the creation of detailed costing proposals for your product development program. Increasing scale, simplifying formats, designing and employing more automation and tooling, and providing purchasing commitments are all potential ways to reduce product costs. Typically, the most cost-effective projects will be those that build upon the development work that has already been incorporated into commercially available lyophilized products. Customization of these products is often a straightforward and low-risk process. Additionally, packaging that enables higher throughput in the dryer and automated sealing (e.g., glass vials) will often represent an economical choice.

For *in vitro* diagnostics customers, license agreements can be crafted to transfer process and/or provide dried product intermediates that can be used to assemble final products in your own facilities.

CAPACITY & DISTRIBUTION

The NEB Lyo Sciences facilities are located in the UK, approximately 2 hours from London. Delineated areas keep quality control processes separate from research and development and manufacturing processes. The facility has the capacity to deliver millions of assays and can work with you to meet your needs at higher volumes. NEB Lyo Sciences is further supported by the NEB global network with headquarters in the US (near Boston, MA) and hubs in Europe and Asia Pacific (Frankfurt, Singapore) to ensure that your product is shipped wherever you need

Experience the benefits of working with a combined organization that will simplify the process of lyophilizing nucleic acid amplification reagents.

To get started, contact us at www.neb.com/CustomContactForm.

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