Questions & Answers

Lyophilization Webinar - Q&A

A Lyophilization Webinar that's 'Not-So-Dry': the Benefits, Challenges, and Best Practices of Freeze-Drying PCR Reagents was held on November 8, 2023. Here is a comprehensive compilation of all the questions raised during the webinar, along with their detailed answers.

1. You mentioned lyo-compatible, lyo-ready and lyophilized products, do you offer products in all three formats?

We currently provide off-the-shelf products that are lyo-ready, such as our "PlexTaq 5x qPCR Multiple Master Mix" and lyophilized products like our "DirectBlood Genotyping PCR Kit" and "qPCR Probe LyoBeads".

If you browse our offerings and cannot find the exact format you're looking for, we are always open to creating tailored solutions and can develop customized products that meet your specific needs.

2. You mentioned challenges you face in the talk, what about humidity?

Ensuring the stability of freeze-dried products against moisture is a key aspect of our operations. This is achieved using specialized lab equipment and airtight secondary packaging. Freeze-dried products, whether in 96-well plates or strips, are stored in aluminum-sealed bags, designed to be opened just prior to use.

When handling and dividing large quantities of freeze-dried products, controlling humidity is crucial. We advise performing these tasks in air-controlled laboratories where low air humidity can be achieved and handled. For this purpose, at Medix Biochemica, we have invested a significant amount in our lab infrastructure to guarantee such conditions so that the quality of our freeze-dried and air-dried products is not compromised.

3. Lyophilization has been around for a number of years now, where do you see this area going in the future? What improvements will be made in this field?

Although lyophilization has been around for a number of years, the significance of lyophilization has surged, particularly in the context of preserving PCR reagents amid the COVID pandemic in recent years. This technique is increasingly vital for point-of-care testing in remote locations, and among personnel with limited training. Therefore, we believe that it is poised to gain even greater popularity and become more relevant in the foreseeable future.

4. Do you have any lyophilized product suitable for the point of care?

We currently sell a spectrum of products which can in principle be used for point-of-care applications. One remarkable product which comes to mind is called Isotherm3G, a mutant Bst polymerase that simultaneously carries out reverse transcription and isothermal amplification and therefore allows for quick and efficient LAMP and RT-LAMP. We are working with some customers at the moment to customize this product to be lyo-ready, with the aim of producing LyoBeads that can be used on POC devices.

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5. I am interested in also lyophilizing a positive control in my mix, is this possible?

Yes, it seems this question is regarding templates, specifically larger oligonucleotides compared to the usual primers and probes. Based on our experience we can safely assume that templates can be included in lyophilized products.

Additionally, it is worth mentioning that most of our products have a hot-start formulation which renders the enzymes inactive at ambient temperatures. This is important to know as we can add primers, probes as well as positive controls before freeze-drying.

Therefore, there should be no problem in adding them.

6. How can I increase my moisture less if I only have around 90%

If we understand this question correctly, you wish to decrease air humidity to handle lyophilized products. 90% air-humidity is very high, and the exposure of any freeze-dried products to these conditions will endanger the stability and functionality of the products if they are not used immediately.

One possibility to avoid this is to work in a controlled environment in which lower humidity can be achieved using air dryers. For this reason, at Medix Biochemica, we have invested a significant amount in our lab infrastructure to guarantee such conditions so that the quality of our freeze-dried and air-dried products is not compromised.

7. How do you select the correct combination of sugars (lyoprotectants and bulking agents)? What is the maximum sugars % suitable for freeze-drying process?

Answering this question isn't straightforward; it greatly depends on the specific contents of the mixture.

For instance, if the mixture contains a high concentration of salts, you will certainly need an effective thermal stabilizer and very low shelf and condenser temperatures at the beginning of the primary drying phase. If you're looking to protect a particular enzyme, you will also require a lyoprotectant.

The suitability of ingredients varies widely. Without examining the exact formulation, it's impossible to give a definitive answer.

I would like to suggest that consulting existing literature or studies on similar mixtures can provide a starting point. However, it often involves a process of trial and error. It's crucial to consider how these excipients may interact with the assay's functionality.

Moreover, when discussing the physical stability of the lyophilized product, such as with LyoBeads, the structural integrity post-lyophilization is key—they must maintain their shape and not disintegrate immediately.

Excipients like bulking agents are not only for stabilization but also to ensure the solidity of the lyophilized 'cake' or 'bead'.

So, in summary, there's no simple answer to this—it requires a nuanced and well-researched approach.

8. What is the best way to make your lyophilization faster (shorter main drying)?

It is very much dependent on the specific materials you're working with. There may be room for adjusting the freezing and primary drying steps of your lyophilization process. You could make the freezing step slower, to increase the ice crystal size and thereby induce faster sublimation. Please keep in mind that this process could be critical when we are lyophilizing enzymes that can be damaged by the large ice crystals.

Experimenting with the primary drying phase might be your best bet, although this is intricate work. New technologies are available that can monitor the drying process in real-time. For instance, using specialized probes in the freeze-dryer, or devices that weigh the product during the process, can indicate when the first drying phase has completed based on weight stabilization, allowing you to proceed to the second phase.

Regarding the freezing itself, the rate can affect the size of the ice crystals formed, which in turn influences the sublimation rate during the lyophilization. Adjusting the temperature differential between the product and the condenser might also speed up the process rather than just altering the vacuum levels, which might not be as effective.

What is clear is that this is a hands-on process; theories can guide you, but practical trials are essential. It's a matter of trial and error, where experience can shorten the learning curve, but experimentation is still necessary.

We suggest you start with protocols that have already been successful according to the literature, adapt them to your requirements, and optimize from there.

9. By shipping at room temperature, do you mean room or ambient temperature? What would be the maximum shipping temperature for lyophilized PCR mixes?

We recommend shipping and storing freeze-dried products at standard room temperatures which are usually between 20-25°C. Previously performed stress-tests indicate that higher temperatures (above 35°C) are generally not good for lyophilized products.

It is worth nothing that freeze-dried products can also be kept in a -20°C freezer if needed.

10. What are the main issues when using buffers containing sucrose and ammonium sulfate inside freeze-dried products?

The lyophilization of buffers with sucrose should not cause any complications. However, ammonium sulfate will lead to a decrease in pH after lyophilization which can influence the performance of your active ingredients. Ammonium sulphate is therefore considered a "no-go" component for lyophilization and should be avoided.

11. How to store single test PCR in PCR tubes?

I believe this is referring to single reactions in PCR tubes/plates, i.e. one reaction per tube/well. This is all prepared in the set-up. In the case of LyoCakes, single reactions of master mix can be loaded into the tubes/wells pre-lyophilization and are lyophilized in place. For the case of LyoBeads, single reaction amounts of master mix can be freeze dried into individual beads and then loaded into the PCR tubes/wells after lyophilization.

All our lyophilized products are optimized to be stored at room temperature. The secondary packaging ensures protection from air humidity.

12. If there are different inorganic salts in the sample, is there any concern about the changes in the salt concentration after reconstitution of lyophilized products?

To minimize this risk, make sure that the volume after reconstitution is exactly your intended reaction volume. Therefore, you should take the volume of the lyophilized product into consideration. It is also important that the sample is properly mixed so that all salts are completely dissolved.

For example, our LyoBeads have a rough "dead volume" of 1 µl, so adding 19µl of a liquid (for example the sample) will result in a final rxn volume of 20 µl.

13. What are the advantages from offering LyoBeads vs LyoCakes? Do both have the same stability in the long run?

In the case of LyoCakes, a volume of master mix is loaded into the tubes/wells pre-lyophilization and are lyophilized in place, so you must decide what primary packaging you want to use before lyophilization can start. Primary packaging and product format decisions play a significant role in the process.

For the case of LyoBeads, single reaction amounts of master mix are freeze dried into individual beads and then loaded into the PCR tubes/wells after lyophilization. So, you have the flexibility to transfer and place the beads in different variants of primary packaging.

Both variants have the same stability. But please take into account that aliquoting LyoBeads in the primary packaging you want needs special equipment to protect the LyoBeads from air humidity.

14. Do you typically do formal stability or solubility study for each product, in particular, the rec. antigens or enzymes before launching products to the market?

With every ISO13485 conform product development, stability and solubility studies are carried out as part of the validation process. Research-use-only products may differ from this.

15. What are the main causes of LyoCake shrinkage?

When we talk about collapsed LyoCakes, the reasons for this can be very diverse. I would recommend optimizing the formulation, for example adding more filling reagents. However, it is difficult to give specific advice without knowing exactly what is being lyophilized. Trying to optimize the freezing and primary drying steps may also be helpful here.

16. Can we expect the same rate of amplification in both lyophilized and liquid reagents?

Yes, the amplification rate is usually not affected by lyophilization. However, it has been observed that the fluorescence intensity may decrease following lyophilization.

17. Is UTM suitable with cake formation and stability?

We do not have prior experience with lyophilizing Universal Transport Medium so cannot comment. It would be important to know what the exact composition of the UTM is as different UTMs can differ significantly.

18. How stable is your LyoBeads in POC machine, if it is exposed to atmosphere?

Exposure to the “normal” atmosphere can cause LyoBeads to get wet and the performance and stability to be compromised. It is therefore suggested that transferring of LyoBeads from their packaging to their final destination should be carried out at very low air humidity conditions. For this purpose, at Medix Biochemica, we have invested a significant amount in our lab infrastructure to guarantee such conditions so that the quality of our freeze-dried and air-dried products is not compromised.

Should you have any additional inquiries or need further clarification, please feel free to contact us at mdx@medixbiochemica.com

View the webinar on demand at: medixbiochemica.com/lyophilization-webinar-thats-not-so-dry

Medix Biochemica

Medix Biochemica
Klovinpellontie 3, FI-02180 Espoo, Finland
medix@medixbiochemica.com • www.medixbiochemica.com