

Steve Lewis 00:10

Welcome to Speaking of Mol Bio, a podcast series about molecular biology and its trending applications in life sciences. I'm Steve Lewis, bringing you another episode of our Mol Bio Minutes mini episodes. Today, we welcome Dr. Agn  Alminait , a senior product manager at Thermo Fisher Scientific. Agn  will be talking about ways to make molecular assays stable at room temperature, and specifically about the new Dry-Ready reagents for assay stabilization in RT-LAMP and RT- qPCR applications. This is an interesting topic for all, but especially for those developing commercial applications. And be sure to stay tuned until the end of the episode for a limited time promotional offer. This offer is exclusively for Speaking of Mol Bio listeners, so you won't want to miss it. Stick around until the end of the episode to get all the details.

Agn  Alminait , PhD 01:09

Hi, I'm Agn . I'm product manager for commercial applications, and my focus is on products and enzymes for isothermal amplification and also high-fidelity polymerases for PCR. Taken together, our team consults on all enzymes that span many workflows in molecular biology. From PCR to single cell sequencing and also signal detection after many amplification methods. Our main customers are big and small assay developers working in plant, animal or human fields. They may be in different stages of their research, from the very beginning of their development to commercial stages, and they are all usually looking for high quality and innovative solutions for their work. We engage with our customers from the start of their development, and we can customize our products, composition wise, buffer wise, we can increase or decrease volume or concentration, or we can start developing new solutions from scratch, depending on our customers' needs. In my work, I constantly meet customers that are developing kits that include PCR or isothermal amplification reagents, and they want to be able to store and ship their kits at room temperature. This would save them time, and this would also save their end users time and hassle. It means that they need help with sourcing reagents that can be made room temperature stable and still deliver the reliability and reproducibility that they need. Assays for various applications need stabilization. Today, I will talk about drying enzymes for RT-qPCR and RT-LAMP.

Traditionally, to obtain room temperature stable assays for these applications they would be lyophilized. Lyophilization is well established and trustworthy process which requires an equipment, a lyophilizer. Compared to assay lyophilization, which is a relatively complex and costly process, air drying is simpler and user friendly. Further in this episode, I'll be explaining the differences between these processes, and hopefully this will help you understand the use for both of these processes. Now I would like to briefly tell you how the enzymes are prepared for drying and what is the main difference when compared to lyophilization.

Enzymes for air drying assays are pre-selected by their thermostability characteristics and stabilized in specifically developed excipient formulation. All these characteristics we call Dry-Ready format. Now you can use Dry-Ready reagents and obtain room temperature stable, dry assays without special equipment in two hours using hot air oven set to 50 degrees Celsius. So how does Dry-Ready differ from layer ready and which one to choose? Just like with food preservation many methods exist. Drying has long been known and used, but it was long feared that enzymes when dried would lose their structure and function during the process. That's why only recently, enzyme and assay drying

approaches have emerged. First there was lyophilization, which is a well-established, scalable approach that is going to be used for a long time to come. But Dry-Ready is a new and simpler approach to obtain dry, stable and also functional assays. So let me help you understand the differences and also the similarities between the two approaches.

Talking about Lyo-Ready reagents, these are enzymes in formulation that doesn't contain glycerol, can, in many cases, be adapted for air drying processes as well. This can be done with the help of excipient mix that is specifically developed for air drying. What are excipients I just mentioned? Excipients are non-reactive additives that preserve the enzymes during drying, or lyophilization for that matter, and help maintain the structural and functional characteristics that are so important for the enzymes function. Excipients for both processes can be similar, but they can also be different. The most critical is to use optimal combination and concentration of these additives.

Technically speaking, lyophilization removes water from substances by freezing and then sublimating the ice under reduced pressure. This process helps preserve product integrity, supports longer shelf life, and is widely used to stabilize sensitive reagents. Air drying, technically, is quite straightforward. It is a gentle heating at moderate temperature in a basic ventilated oven. So lyophilization requires dedicated equipment, while air drying is done in a simple heating oven. The result after both processes is similar. However, there is also differences in humidity content. There is more humidity left after the air-drying process. Nevertheless, assays after both processes have to be strictly protected from humidity. Air drying is a fast process. It takes about two hours; however, precautions have to be taken between the drying runs to restrict cross contamination in the same oven. Also, there are volume restrictions for drying. Lyophilization can take up to 24 hours, but it is highly scalable process and can let you handle even big reaction volumes. Final results after both processes is similar-it is a dry room temperature stable assay.

Now that we've covered what air drying is and its similarities and differences relative to lyophilization. Let me now tell you about the first two products that Thermo Fisher Scientific has to offer in this new Dry-Ready format. First, I will talk about the Dry-Ready RT-LAMP Kit, which is a complete solution, a complete set of enzymes and excipients and buffers to develop RT-LAMP-based detection assay and air dry it in hot air oven. Using this kit, one can dry down a LAMP assay, or RT-LAMP assay. The choice on the customer side, we provide all the reagents in this Dry-Ready RT-LAMP set, meaning that there is excipients included, and the guidelines how to use the hot air oven to obtain room temperature stable assay. After using Dry-Ready RT-LAMP Kit, one can obtain room temperature stable assays that will have a shelf life for up to one year. Air drying RT-LAMP assays brings even more flexibility when compared to lyophilization. It enables even small labs or customers in research stages to develop and stabilize LAMP-based assays that are quite a widespread nowadays in point of care and field use. I will not go into explicit details here about the protocol that we include in the product page, so you can find the protocol in the links under the episode.

The second product I want to talk about is Dry-Ready One-Step RT-qPCR Kit. RT-qPCR technique is probably most widely used reaction in pathogen detection. Similarly, to ensure developed assay stability, lyophilization, or now air drying, can be used. The Invitrogen Dry-Ready One-Step RT-qPCR Kit is an innovative solution designed for preparing hot air-dried RT-qPCR assays. Using air drying

makes assay development simpler and also a shorter process. As I was mentioning, it can be done in under two hours. So also as an advantage, RT-qPCR in itself is a double reaction that simplifies the workflow of handling multiple steps separately and combining everything into one, single reaction. Air drying RT-qPCR reaction offers simple way to obtain room temperature stable RT-qPCR reactions. Again, I will not go into the details how to set up the reaction, you can find it in the protocol pages; in the links we provide below this episode.

So to wrap up today, the process of assay stabilization has never been easier. Dry-ready reagents, an excipient mix, and a drying manual offers a complete toolbox for assay developers and provides huge flexibility. Even labs without sophisticated lyophilization equipment can try and develop room temperature stable assays using just a heating oven in under two hours' time. We provide RT-LAMP and RT-qPCR premixed assay formats and also recommendations how to use them for air drying. Moreover, one can dry the enzymes from their own selection, and they can do it separately or in combinations using the excipient mix we have developed. So if you are developing an assay or a kit that needs room temperature stabilization, I encourage you to follow the links in the notes and explore and learn more about how Thermo Fisher Scientific can support and aid your commercialization efforts. So thank you for listening to today's episode and I hope you found it interesting and useful, and this will inspire you to try these air-drying methods in your experiments.

Steve Lewis 13:04

That was Dr. Agn  Alminait , senior product manager at Thermo Fisher Scientific. As always, for these Mol Bio Minutes mini episodes, we recommend that you check out the Episode Notes to find links to the resources that Agn  mentioned today, as well as others.

Now for our loyal listeners, we have a special promo offer. Be one of the first 75 listeners to spend \$500 and get 40% off all eligible reverse transcriptase, plastics, enzymes, and cloning reagents. If you are in the US, enter promo code C A Z D U A when you place an eligible order. That's C A Z D U A, if you're in the United States. And for customers in Canada, you can use promo code C Z 7 F 1 9. This promo is only available until October 27th so act fast. Check out the Episode Notes for a link or visit thermo.fisher.com/sombpromo for more details, that's thermo.fisher.com/sombpromo. Are you interested in other great promotions from Thermo Fisher Scientific? Check out thermo.fisher.com/promotions, that's thermo.fisher.com/promotions Terms and Conditions apply.

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