

**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 your host Steve Lewis, bringing you another Mol Bio Minutes mini episode. This one is all about adventures with RNA and considerations that OEM kit and assay developers need to consider when choosing the components they source for their work. Our expert on this subject is Thermo Fisher's own Agnė Žiupkaitė. I hope you enjoy.

**Agnė Žiupkaitė 00:44**

Hello everyone and welcome back. Let's talk today about 1-Step RT-qPCR assay, focusing on the reverse transcription step and customization capabilities. When you start thinking about research for molecular methods like pathogen detection, virus surveillance, gene expression analysis, or any other molecular methods, what challenges comes to your mind first? Is it reliability, sensitivity, speed, or even possible inhibitors from sample? I do believe that at least one of those concerns popped out recently. If you have never faced those challenges, you are the lucky one.

I will get back to the challenges in a second, but before that, let's guess what makes you successful in molecular assay development. I would like to answer that with a statement that being efficient is a key. The efficiency could be described through faster workflow, optimized solution for molecular development, multiplexing, ease of use and accurate results with minimal failures. Everyone who works in the molecular assay development field have the similar challenges like launching a new product that maximizes the efficiency and outperforms the competition. But before that, you face a challenge of selecting and choosing the reliable raw materials supplier who is flexible in customization. The molecular assay market consists of assay developers that introduce innovative solutions for testing and is a cornerstone to help detect things as easily and early as possible. And you know when you get tested, you want to make sure that the test results are reliable and you can trust them.

For quite some time, instruments for laboratory use have evolved faster than raw materials used in the assays. It became a great challenge to create innovative technologies with old fashioned reagents. This reason became the driver for assay developers to find the most advanced raw material suppliers that could reflect rising user needs. Since we define that one of the most important laboratory needs is efficiency, let's not forget others, like ease of use and ease of storage. All of this could be covered while choosing not only a reliable reagent supplier, but also the one who is focused on developing faster, more specific, more reliable and one, more robust enzyme and reagent options for laboratory needs. One of the main constraints to develop a short and reliable 1-Step RT-qPCR assay was not fast enough reverse transcription enzyme. In a field where assay time is reduced to less than 15 minutes, the main challenge was to find a reverse transcription enzyme that enables the RT step in less than one minute. You might think that one minute is too short, it's not possible, there is no enzyme that exists in the market right now able to do this. Let me tell you this. It is possible. It's doable. And it's Lyo-ready SuperScript III Flash Reverse Transcriptase that enables you to do a reverse transcription step under one minute. Even when molecular assay developers start business relationship with a reliable and innovation-driven reagent suppliers, one's ability to adapt to the laboratory changing and customized needs is a must.

When we are talking about product customization, it could be divided in a couple of categories. The first one is being flexible and easily done customization, like custom fills, label content or even custom label

template. The second category would be more advanced. Like changing the product formulation. If you have a system that is not compatible with particular component, we can remove it. Or let's say you don't like a specific component, we can remove it as well. Increasing the enzyme concentration is another aspect in the same category that could be done quite easily and quite often. The most advanced customization could be enzyme evolution or enlarging the manufacturing scale. Let's dive deeper into each category's example.

Let's start with a simple one. If you are working in a molecular assay development using 1-Step RT-qPCR assay, and you see a challenge not only to find a fast and robust reverse transcription enzyme, but also see challenge in labeling at your own facility, a customization of receiving a product with your desired label, including the logo might be a solution. We, as a raw material supplier and partner, support you with customization like this. If your goal is not only to shorten the assay time, but also to use reverse transcriptase as your product for commercial kit, we can offer the latest commercial product, like Lyo-ready SuperScript III Flash Reverse Transcriptase, and offer customized label with your logo and information.

When it comes to the RT-qPCR solution, it would be a crime not to reflect on the SuperScript product family that has proven its leadership. The development and improved features started with SuperScript II Reverse Transcriptase. At the time, it was a superior enzyme, and it started the historical heritage of robustness, reliability and flexibility. Later on, SuperScript III Reverse Transcriptase launched and evolved with even more advanced features like increased half-life and improved thermal stability. The last advancement was trend-setting SuperScript IV Reverse Transcriptase, which further evolved the molecular landscape with features like inhibitor resistance, processivity and reaction speed. Offering SuperScript products as one solution allows you to incorporate it into your assay or kit to provide exceptional performance. You can even get this provided to you in a ready-to-use and ship containers with your customized label. This means you don't have to worry about the challenges of creating a labeling facility on your own. Investing time and money in long term development, which might be difficult. So do you feel how your life could already get easier by choosing a reliable raw material supplier, which enables you to customize all the way through the development.

Okay, let me share another example with you. With growing advanced technologies, laboratories are looking for a way to minimize the reaction size. For example, if you are working with cartridge-based technologies, you might face an issue of narrow flow channels, a strictly defined volume for a master mix, and the need to lyophilize your reagents. To ensure that reagents have low viscosity you might consider Lyo-ready formulation which offers enzymes without the glycerol in the composition and they also provide low viscosity. You might ask me, how the enzyme without the crucial component glycerol could work in the assay. Let me answer you that we have a R&D team that works wonders in the development and they manage to overcome the challenge of glycerol removal and create the composition for glycerol-free enzymes that have the same features as the traditional enzymes with a glycerol in it. If you have a limited volume for a master mix in the cartridge, customization can also be done. You might be interested in the higher concentration enzyme, which will allow you to use low volume enzyme in the reaction while not sacrificing efficiency.

Now that we looked at the two examples, let's look beyond these considerations and look more holistically in the challenges that might come. While the customization of RT-based molecular assay

can vary significantly based on the specific application, several overarching challenges and considerations are universally relevant to all RT-qPCR assay development processes. These higher-level considerations include reaction efficiency, which impacts the accuracy and reliability of the assay which are affected not only by the RNA template, but also by the reverse transcription enzyme. For example, if you have a complexly structured RNA and you are running 1-Step RT-qPCR, you need to make sure that the RNA is fully unfolded and cDNA synthesis is done in high accuracy. To make sure that the RNA unfolding process is successful, you need to increase the RT step reaction temperature. And you always should pay attention that your selected reverse transcription enzyme is compatible with higher reaction temperatures. The sensitivity and specificity of the assay, which are crucial for detecting low abundance targets and distinguishing between closely related sequences. Additionally, factors such as sample quality, reagent selection, and data analysis methods play crucial roles in the overall success of the assay. Addressing these universal challenges is essential for developing robust, reliability, and a couple of times mentioned, we can say it together, efficient RT-qPCR assay.

In summary, proper handling of RNA and cDNA synthesis is one of the most important steps to ensure that further molecular applications provide the accurate results. One reverse transcriptase does not fit all the possible applications. So not only is choosing the right one is important but also selecting a supplier who is willing to customize and help contribute to your success. Another very important consideration that is sometimes missed is to make sure that all products that laboratories are used for a new, commercially available product development have the right commercial rights. This is to say that many of the products you might buy are for Research Use Only, which means that you cannot put them into a commercial kit or assay and resell it if you don't have the commercial rights addressed. We encourage you to consider this and talk with your raw material supplier to discuss this and any customization of the product you might need. Working with a supplier that can provide commercial rights is an added value and should be considered while choosing a raw material supplier. The RT itself is not going to do your desired result for 1-Step RT-qPCR assay development. So don't forget to choose DNA polymerase, dNTPs that could give you desired outcome. Not to mention that when 1-Step RT-qPCR is not the only technology and method for molecular diagnostics, so you might consider isothermal amplification enzymes, or maybe you need a modified enzymes to your assay with the commercial rights and needed support for the laboratories. To sum up the full workflow solutions are provided as standalone components. But you don't need to worry about it, since we are offering the optimized solutions with the protocol, not only to give you a flexibility of tweaking your assay, but also to ensure that your development is as fast and smooth as possible.

**Steve Lewis** 13:56

That was Agnė Žiupkaitė, Product Manager at Thermo Fisher Scientific. As always, for these Mol Bio Minutes episodes, we recommend that you check out the Episode Notes to find links and helpful resources that Agnė covered today. Our schedule continues with a full interview in about two weeks, and then another Mol Bio Minutes episode next month. I encourage you to subscribe to get these new episodes as they drop. And until then, cheers and good science. Speaking of Mol Bio is produced by Matt Ferris, Sarah Briganti and Matthew Stock.