SOLIS BIODYNE

INNOVATION POWERED **BY NATURE**

SolisFAST® inhibitor tolerance:

robust performance and accurate target detection even under the most demanding conditions

SolisFAST® range features

- **F**ast – delivers results 2-4x faster
- **A**ccurate – reproducible quantification of up to 5- plex assays with probe-based mixes
- **S**ensitive – consistent results with low- and high-copy targets
- **T**rustworthy – increased room temperature stability up to 6 months adds security and flexibility

For more information, click **HERE**.

PCR inhibitors prevent the amplification of DNA during the PCR, usually by either interacting with DNA or DNA polymerase, which may affect the sensitivity of the reaction or lead to false negative results [1]. To avoid this, samples are generally filtered and DNA is purified [1]. Unfortunately, it is not always possible to remove the inhibitor(s) completely. Once this happens, the inhibitors can bind to the DNA, preventing access to it [1]. They can also reduce availability of cofactors or interfere with their interaction with DNA polymerase [1]. Therefore, a good inhibitor-tolerant PCR mix is very useful to avoid these kinds of inhibitor related problems.

Solis BioDyne's SolisFAST® DNA Polymerase is an *in silico* designed enzyme with fast extension rates and a programmed tolerance to inhibitory substances. Combining that with an optimized buffer, the SolisFAST® qPCR Mixes enable robust qPCR performance and accurate target detection under demanding conditions. Together with our Stability TAG technology, the mixes are not just inhibitor tolerant, but also stable at room temperature for up to six months, depending on the product [2][3][4].

Inhibitor tolerance test

Regarding the positive feedback from our partners, SolisFAST® products were tested to determine whether they can show good inhibitor tolerance with faster than average cycling protocols. To assess the inhibitor tolerance of our SolisFAST® probe-based qPCR mixes, a test system was created and the impact of individual inhibitors, mainly found in PCR reactions, was evaluated.

		Solis BioDyne		Gold standard Inhibitor tolerant competitors		
Source	**Inhibitor**	**SolisFAST® Probe qPCR Mix (no ROX)**	**SolisFAST® Probe qPCR Mix (no ROX) With UNG**	**Competitor Q**	**Competitor M**	**Competitor B**
Urine	Urea	**1.7 M**	**1.7 M**	1.2 M	1.4 M	< 1.2 M
Plants	Pectin	**1.6 mg/ml**	1 mg/ml	1 mg/ml	0.7 mg/ml	1 mg/ml
Sample prep.	DMSO	**11 %**	8 %	8 %	**11 %**	8 %
Sample prep.	NaCl	**150 mM**	130 mM	110 mM	140 mM	< 90 mM
Sample prep.	PBS (1x, pH 7.2. 7.4)	**30 %**	**30 %**	**30 %**	20 %	20 %
Sample prep.	EtOH	**6 %**	5 %	4 %	**6 %**	**6 %**
Soil	Humic acid	**1.4 ng/µl**	**1.4 ng/µl**	**1.4 ng/µl**	**1.4 ng/µl**	**1.4 ng/µl**
Blood	Hematin	3.9 µM	3.9 µM	**4.1 µM**	**4.1 µM**	3.9 µM
Total reaction time on Bio-Rad CFX96		**47 min**			**1 h 7 min**	

Table 1. Inhibitors from different sources and test results of the tolerance study. To assess the inhibitor tolerance of the SolisFAST® probe-based qPCR mixes, a qPCR test system, targeting a 72 bp region of human gDNA, was developed and the impact of common PCR inhibitors to the reaction was evaluated. Three well known inhibitor-tolerant competitor products were also assessed in the same panel. Tolerance limits, meaning the inhibitor concentrations at which the Ct value does not increase by more than 1, are displayed in the table. Fast extension rates of the SolisFAST® DNA polymerase enable shorter run times while still showing strong inhibitor tolerance compared to the "gold standard" inhibitor-tolerant competitor products.

Why were these inhibitors chosen?

Tolerance to **urea** is beneficial for diagnostics of UTI-s (urinary tract infections), STD-s (sexually transmitted diseases) and also fecal samples. **Pectin** is a common inhibitor in plant samples. **DMSO** is often used in sample pre-treatment steps and as a PCR enhancer. To get the most out of the positive effects of using DMSO, the PCR performance itself should not be inhibited by it. Therefore, tolerance to DMSO is an important feature. **NaCl** and **PBS** are also common inhibitors from sample preparation steps. Our performance is on par with competitors in the tests with **ethanol**, which can easily become a contaminating agent to a PCR reaction, as it is often used in sample preparation and DNA extraction processes. **Humic acid** is a challenge with soil samples, as well as environmental, sewage, water and sometimes plant samples. Last but not least, **hematin** may cause problems in analysis of blood samples.

Real-life application possibilities

As previously mentioned, our inhibitor-tolerant products have not gone unnoticed as many of our partners are using these products in their applications with simplified sample preparation methods. We have received good feedback from our partners who have analyzed **dried blood spots**, **urine samples**, **plant leaves**, **seeds**, **soil samples** etc. One good example is our collaboration with MicroGEM where fast and robust extraction methods harmonize with inhibitor-tolerant mixes.

Dye-based qPCR application study by MicroGEM

Application study by MicroGEM using **SolisFAST® SolisGreen® qPCR Mix with UNG**, a qPCR Mix from the inhibitor-tolerant SolisFAST® qPCR range, shows that by combining **MicroGEM's phytoGEM® extraction kit** with Solis BioDyne's fast and inhibitor-tolerant qPCR mix you can go from sample to excellent qPCR results in less than 2 hours, even when dealing with tough samples like **plants**. This sets the bar for more affordable DNA analyses in all those conditions for which the availability of input material represents a limiting factor. You can read more from **HERE**.

Another set of great results comes from **prenatal diagnostics**. As you may know, prenatal diagnostic tests are routinely performed on pregnant women to identify aneuploidy or genetic inherited disorders of the fetus. Fetal DNA that circulates in the maternal blood can be analyzed to identify genetic disorders. Highly efficient DNA extraction is crucial in these settings, because the low abundant material coming from the fetus needs to be efficiently extracted to detect genetic sequences that are not present in the mother.

SolisFAST® SolisGreen® qPCR Mix with UNG (no ROX) was combined with MicroGEM's technology - the **nucleic extractor PDQeX** in combination with the **PDQeX prepGEM® Universal kit**. This resulted in an optimized protocol, which significantly reduces hands-on-time per reaction, plastic consumption, and costs, for extraction of dsDNA from amniotic fluid and maternal blood. Read more about it from the application note **HERE**.

Discussion

The experiments show that SolisFAST® probe-based qPCR mixes performed very well with different inhibitors and based on the results our products are even outcompeting some of the most well-known inhibitor-tolerant products on the market.

The results from our inhibitor tolerance tests are encouraging proof that the products from the SolisFAST® range can be applied to testing different real-life samples. Although the in-house tests were performed with individual PCR inhibitors, good feedback from several clients in real-life applications serves as a strong indicator that a combination of different inhibitors is not an obstacle for the products in the SolisFAST® range.

In conclusion, SolisFAST® products showcased robust performance and great inhibitor tolerance comparable to other top products on the market while being the fastest out of all the tested products.

References

- **[1]** Schrader, C., Schielke, A., Ellerbroek, L., & Johne, R. (2012). PCR inhibitors - occurrence, properties and removal. *Journal Of Applied Microbiology*, 113(5), 1014-1026. doi: 10.1111/j.1365-2672.2012.05384.x
- **[2]** Kahre, O., Artma, K., Kahre, T. (2015). Compositions for increasing polypeptide stability and activity, and related methods (European Patent No. 2501716 B1). European Patent Office.
- **[3]** Kahre, O., Artma, K., Kahre, T. (2016). Compositions for increasing polypeptide stability and activity, and related methods (US Patent No. 9,321,999 B2). U.S. Patent and Trademark Office.
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