



## Everspark

### Questions & Answers

#### Q/ What is the mechanism behind Everspark long-lasting blinking capacities?

The Everspark buffer series rely on a physical deoxygenation system. Contrary to classical enzyme-based buffers, Everspark buffers maintain long-term blinking of your sample in dSTORM microscopy with optimal quality of image reconstruction for several weeks.

#### Q/ How long will blinking be stable for once mounted in Everspark?

Once mounted on your sample, Everspark will maintain blinking phenomenon of common fluorophores in dSTORM microscopy for up to 2 months when using the Everspark buffer and up to 3.5 months when using the Everspark 2.0 buffer. You do not need to replace the buffer in that timeframe.

#### Q/ Which Everspark buffer is most suitable for my application?

Compatible with most fluorophores emitting in the yellow to far-red emission spectrum, use **Everspark** if you are looking for a high quality, long-lasting blinking of your samples in classical one to two-colour dSTORM imaging.

The **Everspark 2.0** buffer works with a wider panel of fluorophores ranging from green to far-red emission, and has also been shown to provide enhanced numbers of far-red dye localizations. Everspark 2.0 will be the best choice for long-lasting blinking in 3-colour dSTORM microscopy experiments, or if you are looking for boosting your spatial image resolution and facilitated image reconstruction.

#### Q/ Which fluorophores is Everspark compatible with?

The Everspark 2.0 allows high resolution dSTORM imaging with dyes ranging from green to far-red emission, while the Everspark buffer works best with yellow to far-red fluorescent dyes.

The following dyes have been tested and approved by the developers and reviewers:

Everspark	Everspark 2.0
JF 549	AF 488
DL 550	MemBright 488
CF 555	FITC
CF/AF 568	FAM
JF 646	Spy555
AF/CF 647	CF 568
Atto 647N	AF 647
DL 650	
CF 680	
Cy5/Sulfo-Cy5	

### Q/ Which fluorophore combinations will be best for multicolour dSTORM in Everspark ?

Up to 2 colours can be imaged sequentially in the Everspark buffer, while 3-colour dSTORM microscopy can be performed in the Everspark 2.0.

Everspark	Everspark 2.0
<p>We generally recommend <b>AF 647</b> (can also be replaced by <b>CF 647</b> or <b>DL 650</b>) + <b>DL550</b> (can also be replaced by <b>CF 568</b>) when doing dSTORM sequentially. Other researchers also validated the following combinations:</p> <ul style="list-style-type: none"> <li>● <b>JF 646 + CF 555</b></li> <li>● <b>AF 647 + JF 549</b></li> </ul>	<p>So far, the following combinations have been successfully used for 3-colour dSTORM microscopy:</p> <ul style="list-style-type: none"> <li>● <b>AF 488 + CF 568 + AF 647</b></li> <li>● <b>FITC + CF 568 + AF 647</b></li> </ul>

### Q/ What super-resolution microscopy techniques are compatible with Everspark ?

On top of dSTORM, Everspark is also compatible with the following SRM techniques:

- PALM
- MINIFLUX
- HILO & TIRF modes

### Q/ Which kinds of imaging vessels can I use Everspark with?

The sealing protocol for Everspark can be easily applied to most commonly used imaging vessels, including 35 mm round petri dishes, depression slides or chambered coverslips. Check our website for detailed protocol videos showing how to seal your sample in different vessel types.

### **Q/ What method is best to seal my sample?**

Everspark vials are provided with a dual-reagent sealing kit we strongly recommend to use. It is the best around and will increase the longevity of your samples once mounted. We also provide you with detailed video protocols on our website showing how to seal your samples after mounting them with Everspark.

### **Q/ How long will it take to seal my sample with Everspark?**

When following our protocol of use, the total time necessary to mount and seal your sample in Everspark should not exceed 5 min. For optimal results, we recommend leaving at least 4 h between sample mounting and image acquisition.

### **Q/ How many experiments can I carry out with one kit of Everspark?**

One kit of Everspark contains 10x tubes of 450  $\mu$ L, providing enough buffer to mount 10x 35mm petri dishes. Volumes can be adjusted depending on the size of the imaging vessel used.

### **Q/ Can I mount several samples at the same time?**

While it is possible to mount several samples at the same time, minimizing the time spent between opening the Everspark vial and sealing the sample will guarantee maximal efficiency and long-term blinking longevity. Once the Eversark vial is opened, we recommend sealing the sample within 1 minute to minimize reoxygenation of the buffer.

### **Q/ What happens if I take more than 1 minute to seal my sample?**

We guarantee optimal blinking efficiency over 2 months for Everspark 1.0 and 3.5 months for Everspark 2.0 when sealing your sample within 1 minute after opening the Everspark vial. Extending the sealing time for up to 30 minutes should not have a significant impact on blinking efficiency when imaging over 3 weeks. In that case however, we cannot guarantee that blinking will remain stable on a longer time frame.

### **Q/ How long can I use the Everspark vials for?**

The Everspark vials can be used up to 6 months when stored at 4°C in their closed pouch.

### **Q/ Which reagents & equipments do I need to use Everspark?**

The Everspark kit contains ready-to-use Everspark blinking buffers, and the required reagents to seal your sample. All you need to have on your end is your imaging vessel of choice, a coverslip and 1mL syringes. For green fluorophore dSTORM imaging in Everspark 2.0, the 488 nm laser should have sufficient power to get a high quality image in the green channel. We recommend using a laser

>200mW, ideally 500mW for optimal results.

**Q/ I am experiencing photobleaching when performing dSTORM imaging in Everspark buffer.**

Photobleaching is usually related to a reoxygenation of the buffer, so properly sealing your sample is critical to avoid it. Some key elements to consider when sealing the sample in Everspark:

- Seal as quickly as possible after opening up the vial (ideally <1min).
- Make sure the sealing is homogeneous and that no open areas remain.
- Make sure there are no air bubbles trapped in the buffer.

*Last updated in January 2024.*