Everspark

Protocol

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1. The material you need

Reagents

- 1x 450µL Everspark vial (in the kit)
- Blue and yellow sealant (in the kit)

Consumables

- 1x WillCo-dish® Glass Bottom dish or 1x depression slide*
- 1x round 25 mm coverslip
- Kimwipes™
- Tweezers
- 3x syringes
- 20G x 1/2" size needle (optional)

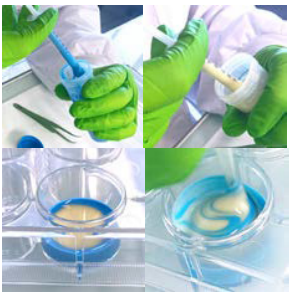
***We recommend using either WillCo-dish® Glass Bottom dishes or depression slides as this sealing method has been validated in both vessel types.**

2. Important notes

- Unopened Everspark vials can be stored at 4°C for up to 6 months when stored in their closed pouch.
- Once mounted in Everspark buffer, blinking of fluorophores will remain stable without any loss of events or photons per events for up to 2 months when using Everspark 1.0, and 3.5 months when using Everspark 2.0.
- Mounted samples can be imaged repeatedly over these time frames when stored at 4°C protected from light.
- When using Everspark 2.0, the 488 laser sould have sufficient power to get a reliable image in the green channel. We recommend using a laser >200mW, ideally 500mW for optimal results.

3. Mounting samples with Everspark

- Rinse the WillCo-dish® Glass Bottom dish or depression slide with 1-2 mL of sterile water.
- Discard all the water in excess.
- Prepare the sealant:
Take 1 mL of sealant catalyst (blue) with a syringe + 1 mL of sealant basis (yellow) with another syringe.
In a microdish, mix them with a tip.



- Centrifuge the Everspark vial for a few seconds.

The following steps should be performed as quickly as possible to minimize buffer reoxygenation.

Once the Everspark vial is opened, the sample should be sealed within 1 minute to guarantee a stable blinking in time (2 months for Everspark 1.0, 3.5 months for Everspark 2.0).

- Open the Everspark vial and pipet the adequate volume right away in your vessel (i.e. 450µL for a WillCo-dish® Glass Bottom dish).

Although several depression slides may be mounted with a single tube of Everspark, keep in mind that all steps from opening the vial to sealing should take no more than 1 minute.

The buffer should form a dome in the dish to avoid the formation of bubbles afterwards.

• Delicately place the coverslip in the cavity filled with Everspark.

Make sure the coverslip covers properly the whole cavity and remove any bubble that formed as they are a source of oxygen.

• Absorb any excess of the Everspark buffer on the sides with a Kimwipe™.

• Press very softly with tweezers on the coverslip to remove the bubbles.

• Take up the prepared sealant in a fresh syringe and quickly seal the sample by rotating the WillCo-dish® Glass Bottom dish.

Adding a compatible needle to the syringe will facilitate sealing and improve precision, especially when working in depression slides. We recommend using needles with a 20G x 1/2" size to avoid clogging issues.

• Wait for 3 minutes until the sealant is polymerized.

Check that the sealant is polymerized by checking in the syringe or the microdish.

• Discard the open vial of Everspark buffer. Once opened it must not be reused for another slide.

There should be no bubble.

4. dSTORM imaging with Everspark

• Wait for 4h before imaging for optimal results.

• Gently clean the WillCo-dish® Glass Bottom dish with 70% ethanol just before the acquisition.

Compatible fluorophores:

Everspark 1.0 works best with yellow to far-red fluorophores.
Validated fluorophores include JF 549, DL 550, CF 555, CF/AF 568, JF 646, CF/AF 647, Atto 647N, DL 650, CF 680 & Cy5.

Everspark 2.0 is compatible with green, yellow & far-red fluorophores.
Validated fluorophores include AF 488, MemBright 488, FITC, FAM, Spy555, CF 568 & AF 647.

Specific tips for performing 3-colour microscopy with Everpark 2.0:

The highest number of blinking events & best precision are usually obtained in the red/far-red channels.

• Where possible, we recommend using green fluorophores for the largest structures and far-red dyes for structures requiring the highest precision.

• The 488 laser sould have sufficient power to get a reliable image in the green channel. Where possible, we recommend using a laser >200mW or ideally 500mW for optimal results.

• For sequential illumination in 3-colour microscopy, we recommend starting from the highest to the smallest wavelengths to illuminate the sample (i.e. far-red, red then green).

Check out our website for updated information on validated dyes, published studies using Everspark, tips for imaging, FAQ and much more:

idylle-labs.com
30, rue de Campo-Formio
Paris - France
+ 33 1 84 25 51 44
contact@idylle-labs.com

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