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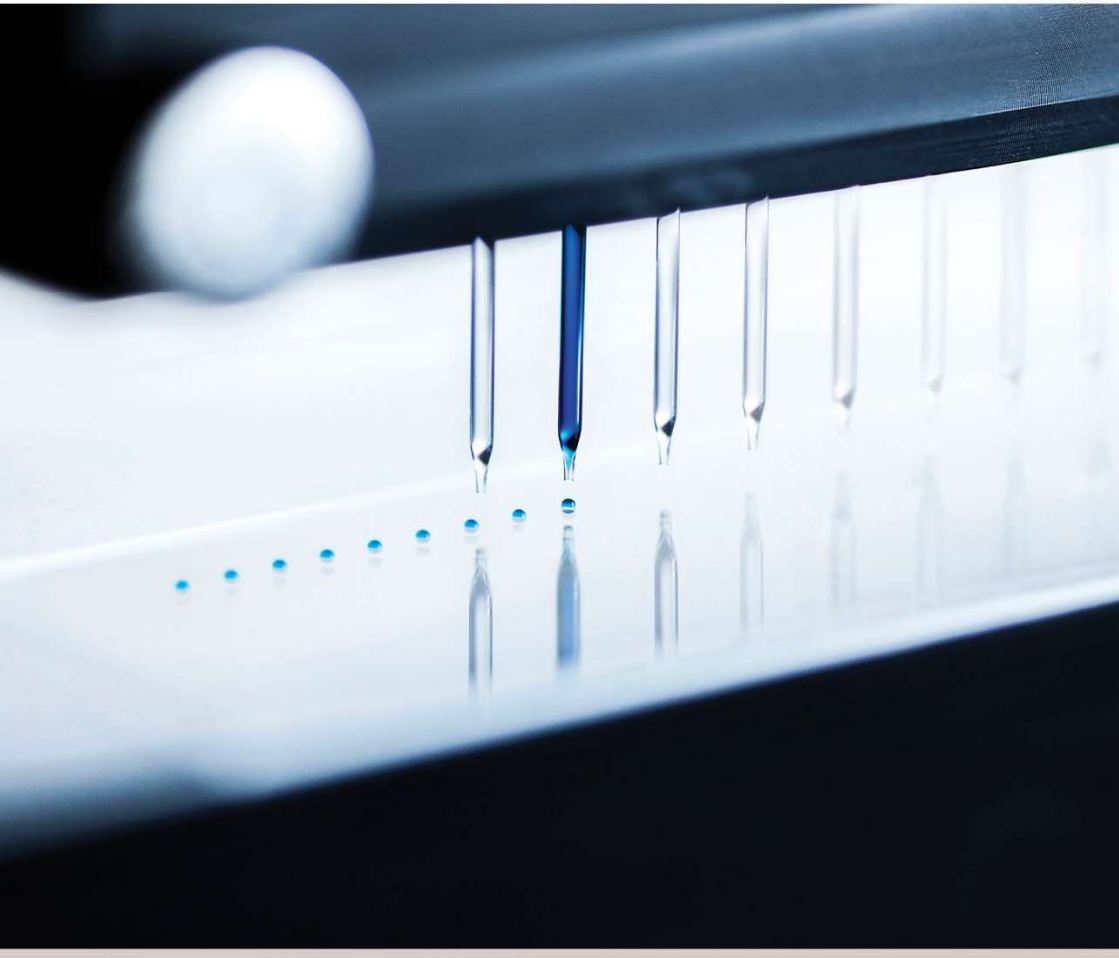

INSTRUCTION MANUAL

sciPOLY 3D

Version 230131

Safety Considerations: When working with sciPOLY3D and SCIENION Buffer System please follow all generally accepted laboratory safety guidelines. At a minimum, wear appropriate personal protective equipment such as a lab coat, safety glasses, powder-free latex gloves, etc.. Follow recommended standard operating procedures for any laboratory equipment used in your experiments.

Product Use Limitations, Warranty, Disclaimer: sciPOLY3D and SCIENION Buffers have been scientifically developed and are sold **For Research Use Only**. sciPOLY3D and SCIENION Buffer System are not for use in human diagnostics or for drug purposes. Extreme care and exact attention should be practiced in the use of the materials described herein. All SCIENION products are subject to extensive quality control and are guaranteed to perform as described when used properly. Any problems with any SCIENION product should be reported to SCIENION immediately. SCIENION's liability is limited to the replacement of the product, or a full refund. Any misuse of this product is the full responsibility of the user, and SCIENION makes no warranty or guarantee under such circumstances.

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Content

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1. Main characteristics

- sciPOLY3D enables immobilization of biomolecules on most polymeric substrates, without the need for functional groups on the surface or the biomolecule.
- sciPOLY3D enables covalent immobilization on protein-repellent surfaces, which reduces background and makes blocking obsolete.
- sciPOLY3D is water-soluble and is added to the printing media.
- After printing, a short exposure with UV light leads to a surface-attached hydrogel with covalently attached biomolecules.

2. Introduction

The sciPOLY3D polymer enables functionalization of surfaces with biomolecules. It is water soluble and can thus be dispensed together with e.g. proteins or DNA probes. It contains a photo reactive moiety, which upon UV irradiation couples the polymer chains to polymeric substrates, crosslinks the polymer chains leading to a polymer network and covalently attaches the biomolecules to the network. Due to the hydrophilic nature of the polymer, a surface-attached hydrogel with covalently embedded probe molecules is obtained.

The substrates do not need pretreatment, any native polymer substrate (e.g. PMMA, COP, COC, PP, PS, etc.) can be used.

We offer several products related to sciPOLY3D; to get started the Starter Kits are a very convenient option to try and test spotting of approx. 40 different samples and conditions.

- sciPOLY3D Protein Starter Kit (Part No.: CP-5807)
- sciPOLY3D DNA Starter Kit (Part No.: CP-5808)

For spotting up to 100 samples, sciPOLY3D LIQUID is recommended, which is readily dissolved.

- sciPOLY3D LIQUID (Part No.: CP-5803-05)

For larger scale spotting we offer sciPOLY3D SOLID. Here you can dissolve the desired amount by yourself.

- sciPOLY3D SOLID (Part No.: CP-5802-10 or -100)

Related consumables are available, for dissolving sciPOLY3D SOLID, sciPOLY3D SOL1 needs to be ordered.
sciPOLY3D SOL2D1 is the buffer of choice for DNA microarrays, sciPOLY3D SOL2P1 is recommended for protein applications. We have further options available, please contact our support.

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- sciPOLY3D SOL1 (Part No.: CP-5804-5 or -50)
- sciPOLY3D SOL2D1 (Part No.: CP-5805-1 or -100)
- sciPOLY3D SOL2P1 (Part No.: CP-5806-1 or -100)
- sciCHIP COP (Part No.: CSP-5312-5)

96 well plates with protein-repellent properties are available on request.

3. Preparation stock solution and storage

Preparation of a stock solution is recommended with 5 mg/ml sciPOLY3D in sciPOLY3D SOL1. Producing a stock solution with 10 mg/ml is also an option, if needed. But be aware that in this condition a precipitate might form together with concentrated sciPOLY3D SOL2D1 buffer. End concentration in the printing media generally is 1 mg/ml.

Last step in the synthesis procedure is the lyophilization of sciPOLY3D, therefore it comes as fluffy flakes, which easily dissolve in water. Vortexing for at least 2 times 2 minutes is recommended to yield a clear solution. The stock solution can be stored for up to 4 weeks, if stored light protected and in a fridge.

4. Applications with oligonucleotides

4.1. General considerations

Oligonucleotides to be used as probes do not need a functional group, such as $-NH_2$. But a 5' extension with 15 thymines is recommended to increase immobilization efficiency. The applied UV dose (1.25 J/cm^2, 254 nm) leads to radicalization of approx. 10% of the thymines, i.e. approx. 1 or 2 additional crosslinks per oligo.

The user is free to choose any concentration of probe oligo, but concentrations higher than 5 µM do not increase significantly the max. signal intensity (together with 1 mg/ml sciPOLY3D and sciPOLY3D SOL2D1), see Figure 1.

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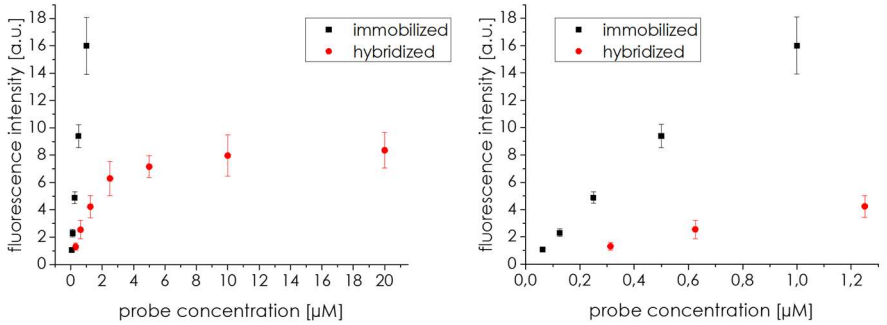

Figure 1 Relation between concentrations of immobilized oligos and fluorescence intensity. Immobilized is a dye-labelled oligo, or an unlabelled oligo, respectively, which was hybridized with a dye-labelled antisense oligo. Printed with 1 mg/ml sciPOLY3D and sciPOLY3D SOL2D1.

4.2. Buffer

The buffer is needed to prevent formation of a “coffee stain” or “donut” and thus leads to a more homogeneous distribution of probe oligos. The sciPOLY3D SOL2D1 (pH=7.4) is the best choice for printing oligos with sciPOLY3D. Increasing the buffer concentration could increase the signal intensities, but be aware that a concentration of more than 1.5x will precipitate together with sciPOLY3D, if the stock solution contains 10 mg/ml**.**

Another possibility is to increase the concentration of sciPOLY3D, while decreasing the buffer concentration, which could improve both signals and stability of the array. That depends on the individual settings, please contact SCIENION, we have different individual options available.

4.3. Temperature

The hydrogel and the oligos are attached covalently to the substrate. Hence, the microarrays can be processed at high temperatures, as needed for example in PCR cycles (up to 95 °C) or melting curve analyses. This feature also enables repeated regeneration and re-use of the microarrays if needed. For more details, you are referred to the Application Notes on our homepage.

4.4. Storage

The produced microarrays can be stored at room temperature and protected from light for several weeks. After approx. 3 months signals might be reduced. The microarrays might still be functional even after several years, but have reduced signal intensities. In general, the shelf life is determined by the biomolecule, sciPOLY3D has no influence on this.

4.5. Exemplary protocol

Preparation of print media

Dissolve sciPOLY3D SOLID in sciPOLY3D SOL1: weigh in the polymer using a precision balance, add appropriate amount of sciPOLY3D SOL1 to yield a stock solution with 5 mg/ml. Mix thoroughly on a vortexer for 2 minutes, wait 5 minutes and repeat mixing on a vortexer for 2 minutes to completely dissolve sciPOLY3D; a clear solution should be obtained. The stock solution should be stored at 4 °C and protected from light, it should be used within 4 weeks.

Prepare a mastermix containing 10 µL sciPOLY3D stock solution and 25 µL sciPOLY3D SOL2D1 for each parameter to be printed. Mix 35 µL of this mastermix with 15 µL of your probe (50 µL total volume). End concentration in the printing solution is 1 mg/mL sciPOLY3D.

	C_{stock}	1 Parameter	
sciPOLY SOL2D1	2x	25 µL	Prepare as mastermix
sciPOLY3D	5 mg/mL	10 µL	
probe	variable	15 µL	
total		**50 µL**	

Notes:

Please avoid additives like betain, glycerol, Tween-20, SDS or any other detergents in the printing solution.

It is recommended to have all spots with the same total concentration of oligonucleotides. If different concentrations are needed (e.g. for controls), add some unspecific oligonucleotide. This ensures the same spot morphology and probe distribution within the spot.

The rel. humidity during printing has an impact on the drying process of the spots. Please make sure to print at a relative humidity below 50 %.

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UV irradiation

Directly after the printing process, place the microarrays in a UV crosslinker (e.g. Stratalinker) with a wavelength of 254 nm, choose energy dose control with 1.25 J/cm2. The time period between printing and crosslinking is not relevant, since a potential minor residual moisture does not interfere with the crosslink process. However, crosslinking should be done the same day.

Washing

Washing the slides with DNA microarray in an ultrasonic bath can be performed to remove all parts that might detach during the assay or following washing steps. Place the slides in a container with 50% ethanol and sonicate for 5 minutes.

Rinse with water and dry.

This procedure is recommended if your downstream processing steps include rather harsh conditions.

5. Applications with proteins

5.1. General considerations

No modification/functionalization of proteins or substrates is needed. Proteins are covalently immobilized in random orientation. Additionally, immobilization by steric entrapment within the polymer network takes place.

In contrast to oligonucleotides, proteins differ a lot in their properties, such as stability, size, charge, hydrophobicity… Thus, there might be optimization necessary of parameters like concentration, storage, best buffer, applicable UV dosage and wavelength etc. Please contact SCIENION for support, if needed.

Be aware that it has been observed that immobilizing proteins with sciPOLY3D could decrease their functionality as compared to simple adsorption. That depends on the protein species. However, simple adsorption is not always an available option.

5.2. Buffer

Most protein arrays with sciPOLY3D so far were printed with sciPOLY3D SOL2P1. Additives like sciSTAB S3 (Part No. CD-6501-2) can increase homogeneity and do not interfere with immobilization, but detergents must be avoided in the printing solution. Some protein storage buffers contain glycerol, which causes problems with spot stability.

sciPOLY3D SOL2D1 was also tested successfully as printing buffer. The choice of suitable printing buffers depends on the individual protein.

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5.3. Storage

There is no general statement about storage, this depends strongly on the individual protein. It is recommended to store the microarrays in the fridge (4 °C). It has been shown that protein extracts (for allergy tests) immobilized with sciPOLY3D were functional for at least one month, independent of storage temperature (4 °C, 25 °C, 37 °C). Same holds true for IgG antibodies. In general, the shelf life is determined by the biomolecule, sciPOLY3D has no influence on this.

5.4. Exemplary protocol

Preparation of print media

Dissolve sciPOLY3D SOLID in sciPOLY3D SOL1: weigh in the polymer using a precision balance, add appropriate amount of sciPOLY3D SOL1 to yield a stock solution with 5 mg/ml. Mix thoroughly on a vortexer for 2 minutes, wait 5 minutes and repeat mixing on a vortexer for 2 minutes to completely dissolve sciPOLY3D; a clear solution should be obtained. The stock solution should be stored at 4 °C and protected from light, it should be used within 4 weeks.

Prepare a mastermix containing 10 µL sciPOLY3D stock solution and 25 µL sciPOLY3D SOL2P1 for each parameter to be printed. Mix 35 µL of this mastermix with 15 µL of your probe (50 µL total volume). End concentration in the printing solution is 1 mg/mL sciPOLY3D.

	C_{stock}	1 Parameter	
sciPOLY SOL2P1	2x	25 µL	Prepare as mastermix
sciPOLY3D	5 mg/mL	10 µL	
probe	variable	15 µL	
total		**50 µL**	

Notes:

Please avoid additives like betain, glycerol, Tween-20, SDS or any other detergents in the printing solution.

It is recommended to have all spots with the same total concentration of protein. If different concentrations are needed (e.g. for controls), add some unspecific protein, like BSA. This ensures the same spot morphology and probe distribution within the spot.

The rel. humidity during printing has an impact on the drying process of the spots. Please make sure to print at a relative humidity below 50 %.

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UV irradiation

Directly after the printing process, place the microarrays in a UV crosslinker (e.g. Stratalinker) with a wavelength of 254 nm, choose energy dose control with 1.25 J/cm2.

If the crosslinking of relevant epitope(s) of the printed protein is an issue at this wavelength, a crosslinker with 365 nm should be used. The energy dose should be increased to 16 J/cm2). The time period between printing and crosslinking is not relevant, since a potential minor residual moisture does not interfere with the crosslink process. However, crosslinking should be done the same day.

6. Suitable substrates

In general, all substrates with C-H moieties are suitable for immobilization with sciPOLY3D. For example, all common plastics like PMMA, COC, COP, PP etc. can be used.

Substrates should be clean and free of residuals, but do not need further pre-treatment or modification and may be used just as they are.

Note: Spot diameter might vary, dependent on the contact angle of the substrate.

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7. Ordering information

Product	Order No.
sciPOLY3D SOLID (10 mg)	CP-5802-10
sciPOLY3D LIQUID (0.5 mL)	CP-5803-0.5
sciPOLY3D SOL1 (50 mL)	CP-5804-50
sciPOLY3D SOL2D1 (1 mL, 2x conc.)	CP-5805-1
sciPOLY3D SOL2D1 (100 mL, 2x conc.)	CP-5805-100
sciPOLY3D SOL2P1 (1 mL, 2x conc.)	CP-5806-1
sciPOLY3D SOL2P1 (100 mL, 2x conc.)	CP-5806-100
sciPOLY3D DNA Starter Kit	CP-5808
sciPOLY3D PROTEIN Starter Kit	CP-5807
sciCHIP COP (5 slides)	CSP-5312-5

For ordering, please visit shop.scienion.com or contact us via mail ticket@scienion.com or phone +49 (0)30 63921700.