

Functionalized Microplates



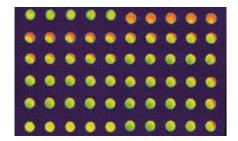


About PolyAn

PolyAn is a nanotechnology company specialized in the modification of surfaces using Molecular Surface Engineering (MSE). Since 1996 PolyAn develops and manufactures high-performance consumables for multiplex diagnostics and LifeScience research.

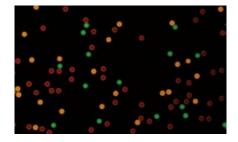
Functionalized Surfaces for Microarrays

PolyAn is one of the leading producers of functionalized substrates for microarrays. Our wide range of surfaces, substrates, and handling tools for microarrays enables our customers to select the most suitable substrate for their specific application.



Microparticles & Submicron Particles

PolyAn is offering a portfolio of monodisperse polymethyl methacrylate (PMMA) microparticles (beads) for multiplex bead assays, calibration of flow cytometers, and calibration of fluorescence imaging systems. PolyAn's microparticles can be color encoded with a wide range of fluorescent dyes and functionalized with PolyAn's reactive 3D-matrices.



Functionalized Microplates

PolyAn's microplates are used for the covalent binding/conjugation of biomolecules that cannot be immobilized efficiently by passive adsorption. PolyAn offers Amine-binding, 3D-Azide and Streptavidin/Neutravidin-coated 96-well plates for challenging ELISA applications.



Calibration Tools

Re-usable calibration tools for fluorescence based detection systems. PolyAn's calibration slides for cell assays can be used as quality controls in a number of in vitro diagnostics (IVD) systems for immunology applications.



Molecular Surface Engineering Services

PolyAn is able to equip almost any substrate with our reactive matrices for selective immobilization and with antifouling surfaces for the reduction of cell adhesion and non-specific binding, respectively. As part of our Molecular Surface Engineering services, we offer functionalized consumables for OEM applications, which are tailored to specified customer requirements.



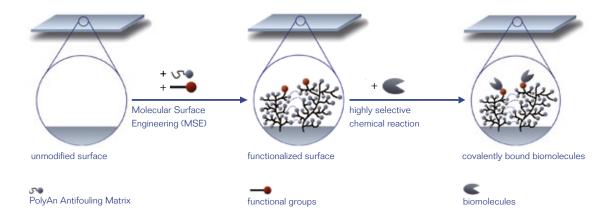
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1. Molecular Surface Engineering (MSE)

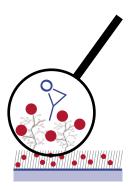
3D-Surfaces for high-performance Applications

PolyAn's Microplates are functionalized with a 3D-Surface chemistry comprised of a long chain polymer containing a homogeneous density of reactive functional groups. The 3D-reactive Surfaces can be applied for the covalent attachment of biomolecules such as proteins, peptides, and oligonucleotides:



Functionalized Microplates for Advanced Bioassay

PolyAn's functionalized Microplates are optimal for immobilizing biomolecules that inefficiently coat by passive adsorption. Select the most suitable combination of surface and assay conditions for your specific assay from our broad range of different reactive groups.



Key features:

- Reactive surfaces for covalent immobilization of biomolecules ensure minimal leaching
- Withstands rigorous washing
- Minimal non-specific binding due to 3D-functional matrix
- Directed (bio-orthogonal)
 immobilization via click chemistry

Applications include:

- Covalent binding of peptides, glycans, oligonucleotides, aptamers, or small molecules for bioassays
- Immunoassays such as Enzyme linked immunosorbent assays (ELISA) and Fluorescence-based immunoassays (FIA)
- Printing of microarrays into 96-well plates

2. Functionalized Microplates

To take advantage of the existing liquid handling and automation solutions for 96-well standard microplates, PolyAn offers a range of functionalized multiwell products that are equipped with the same reactive surfaces as our glass and polymer slides.

96-Well Microplates

PolyAn offers functionalized 96-well plates with various plate designs, including:

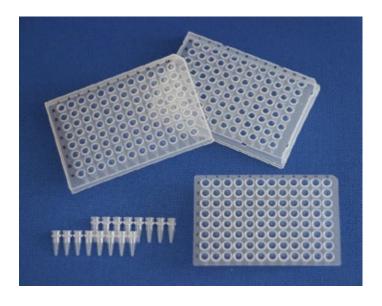


- Standard ELISA plates
- Breakable well plates
- 12 x 8-Well strips and Single-wells
- Polystyrene (PS), Polypropylene (PP),
 Cyclic olefin copolymer (COP)
- F-bottom, C-bottom, U-bottom, μClear ...



PCR Plates

Polymerase chain reaction (PCR) is a method widely used to rapidly make up to billion copies of a specific DNA sample. The technology is used in applications from basic research to high-throughput screening. To address a growing number of inquiries for reactive PCR plates that enable covalent and even bio-orthogonal immobilization of aptamers, oligonucleotides, peptides, and other biomolecules, we are now offering functionalized PCR plates.



Glass bottom plates

PolyAn's multipart plates are comprised of a functionalized bottom plate (75 mm x 110 mm) which can be combined with a superstructure after the printing process. This approach increases the printing throughput while minimizing errors due to electrostatic interactions or geometry.



Bottom materials

- Standard 1 mm Glass sheets
- 0.17 mm Coverslips (Type #1.5, #1.5H, ...)
- 1 mm PMMA

Surfaces

- 2D-Epoxy, 2D-Amine, 2D-Azide
- 3D-Epoxy, 3D-NHS, 3D-Aldehyde, 3D-Amino
- Streptavidin, Neutravidin

Solvent-stable Polypropylene Plates

PolyAn offers functionalized, solvent stable microplates that are comprised of polypropylene (PP). Our PP microplates are suitable for applications requiring increased chemical resistance, e.g. liquid storage, compound libraries in drug screening, synthesis, and covalent immobilization of (bio-)molecules.

- Polypropylene provides compatibility with many common organic solvents
- Minimal leaching due to covalent immobilization
- Coating with lower amounts of reagent may be possible
- Reactive surfaces enable control of orientation and directed (bio-orthogonal) binding

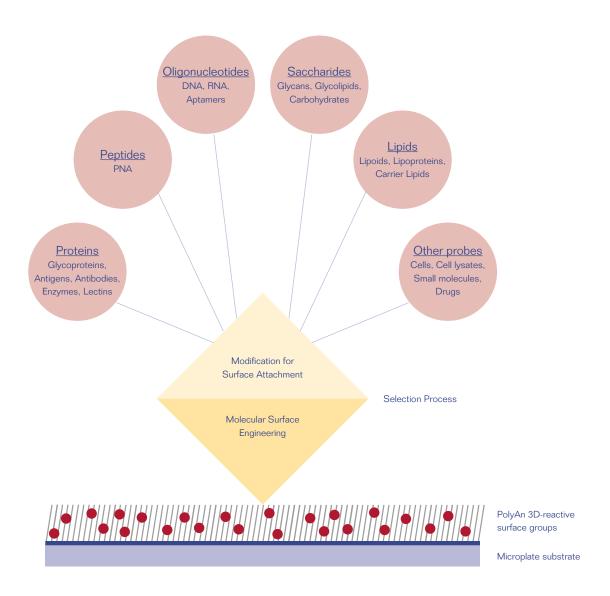
Custom Plates

PolyAn also offers functionalization services for custom (plate) formats upon request. Please contact us for assistance in selecting the correct product for your application or to discuss a custom product development.

3. Reactive Surfaces for Microplates

Functionalized Microplates for Binding of Biomolecules

Selecting the optimal immobilization method for a probe is often an iterative process. For the immobilization of biochemical species various coupling techniques and coupling approaches have been developed. PolyAn offers a very broad portfolio of surfaces and substrates to enable the selection of the optimal surface for each probe and application.



Based on our Molecular Surface Engineering technology PolyAn is offering a range of reactive, functionalized microplates. These surfaces are the ideal platform for the covalent immobilization of biomolecules and allow the selection of the most suitable microplate surface for your specific assay conditions.

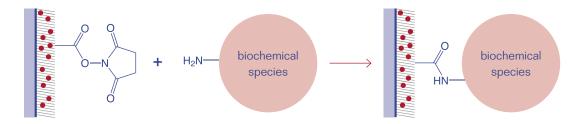
| Functional Group | <u>Structure</u> | Application examples |
|--------------------------------------|---|--|
| 3D-NHS | 0 0 0-N | For direct binding of Amine-containing molecules |
| 3D-Ероху | | For binding of nucleophiles such as Amine-, Thiol-, and Hydroxy-containing molecules |
| 3D-Aldehyde | ——(H | For reaction with Amine- and Hydrazine-containing molecules |
| 3D-Azide | NN≡N | For reaction with Alkyne groups and DBCO-modified molecules via Click chemistry |
| 3D-Dibenzocyclooctyne (3D-DBCO) | | For binding of Azide-modified molecules via Click chemistry |
| 3D-Methyltetrazine (3D-MTZ) | $\begin{array}{c} N=N \\ N-N \end{array}$ | For fast ligation with TCO-modified molecules |
| 3D-Streptavidin or 3D-Neutravidin | | For coupling of Biotin-functionalized molecules |
| 3D-Antifouling | <i>></i> | For low cell binding and/or low protein binding surfaces |

3.1 Covalent binding Microplates

PolyAn's covalent binding microplates are optimal for immobilizing biomolecules that inefficiently coat by passive adsorption. PolyAn offers reactive surfaces that bind amines and other nucleophilic groups, providing a convenient method to covalently immobilize biomolecules, e.g. proteins, peptides, oligonucleotides, glycans, or other ligands.

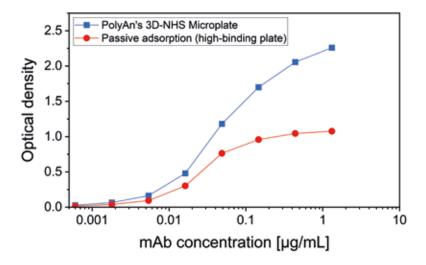
3D-NHS Microplates

provide a highly reactive surface for fast coupling of primary amines.



PolyAn's 3D-NHS Microplates allow a direct and simple approach to covalently attach amine containing molecules to the well surface.

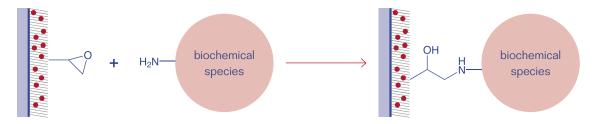
Covalent binding to PolyAn's 3D-NHS Microplates can improve signal intensities and signal-to-noise ratios in enzyme-linked immunosorbent assays (ELISA). In some applications it is also possible to reduce the required amount of antibodies in comparison to passive (adsorptive) immobilization.



Comparison of the PolyAn 3D-NHS surface with a passive/adsorptive binding surface for ELISA applications: The antigen was immobilized/bound to the surfaces, the corresponding monoclonal antibody (mAb) was added at different concentrations, and the immune complexes were detected using HRP-labelled secondary detection antibodies. The amount of mAb required to obtain an optical density (OD) of 1 is 8x less for the 3D-NHS surface compared to a passive binding surface.

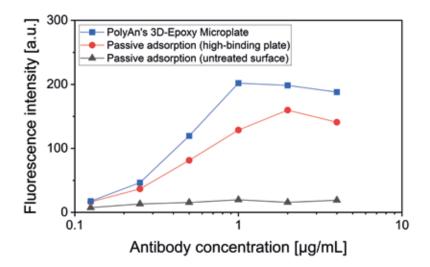
3D-Epoxy Microplates

for covalent immobilization of biomolecules with nucleophilic groups.



PolyAn's 3D-Epoxy Microplates react with nucleophilic groups, e.g. amines, thiols, or hydroxyl groups, to form a covalent bond. The microplates possess an uncharged surface with integrated low fouling matrix and a long shelf life of up to two years.

Covalent binding to PolyAn 3D-Epoxy Microplates can increase the number of immobilized biomolecules (e.g. antibodies), and thus, the resulting signal intensities in the final bioassay (e.g. in fluorescence-based immunoassays, FIA).



3D-Aldehyde Microplates

for covalent immobilization of amine-containing biomolecules.

Aldehyde groups bind to amines to form an imine (Schiff-base) as an intermediate. To increase the bond strength it is possible to reduce the imines with e.g. $NaBH_4$ or TCEP (Tris(2-carboxyethyl)phosphine) to form stable secondary amines.

3.2 Streptavidin/Neutravidin coated Microplates

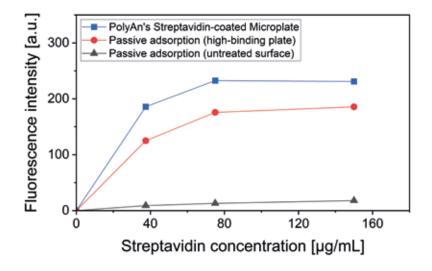
Streptavidin and Neutravidin are tetrameric proteins that can bind four Biotin molecules (vitamin B7) or any other Biotin-conjugated species with a very high specificity. The Streptavidin/Neutravidin-Biotin bond is one of the strongest, non-covalent bonds known in biochemistry, having a dissociation constant of $K_n = 10^{-15} \text{mol/L}$ Thus, it is often applied in bioanalytical applications



Streptavidin- and Neutravidin-coated plates are made using PolyAn's proprietary Molecular Surface Engineering technology to achieve maximum efficient coating of active Streptavidin or Neutravidin. PolyAn's Streptavidin/Neutravidin coated microplates are suitable for immunoassays as well as DNA hybridization assays.

Benefits of covalently bound Streptavidin/Neutravidin

PolyAn's Streptavidin or Neutravidin matrices are covalently attached to the surface, so that the molecules are less susceptible to desorption in the presence of surfactants, solutions of high ionic strength, or at high temperatures, compared to adsorptive immobilization.



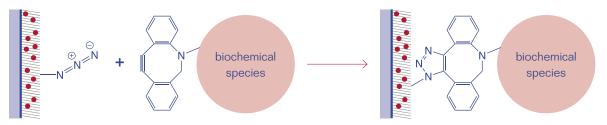
Key features:

- No leaching of Streptavidin/Neutravidin due to covalent immobilization
- Minimal non-specific binding due to 3D-functional matrix
- Ready-to-use: No additional washing or blocking steps required
- Each plate is packed in a separate foil bag to ensure optimal shelf-life

3.3 Reactive Microplates for Click Chemistry

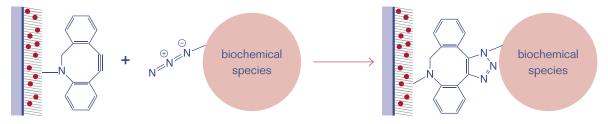
Click chemistry describes quick and irreversible one pot conjugation reactions that have a high reaction specificity, give a high yield of the desired product, and lead to minimal byproducts. Bio-orthogonal reactions are conjugation reactions that do not interfere with biological processes. PolyAn offers a variety of surfaces that are suitable for bio-orthogonal, oriented conjugation of oligonucleotides, peptides, and other small (bio)molecules via click chemistry.

3D-Azide Microplates



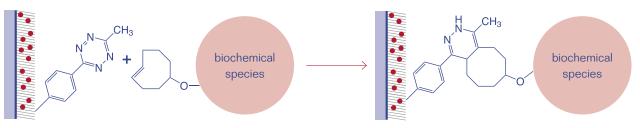
PolyAn's 3D-Azide Microplates can be applied for bio-orthogonal binding of molecules that contain either Alkyne groups in a Copper-catalyzed Alkyne-Azide Cycloaddition, or Dibenzocyclooctyne (DBCO) groups in a Strain-promoted Alkyne-Azide Cycloaddition.

3D-DBCO Microplates



PolyAn's 3D-DBCO (Dibenzocyclooctyne) Microplates can be used to bind Azide-modified molecules via copper-free click chemistry (Strain-promoted Alkyne-Azide Cycloaddition).

3D-MTZ Microplates



PolyAn's 3D-MTZ (Methyltetrazine) Microplates can be applied for the fast and efficient binding of biomolecules that are modified with a trans-Cyclooctene (TCO) group via an Inverse electron- demand Diels Alder reaction.

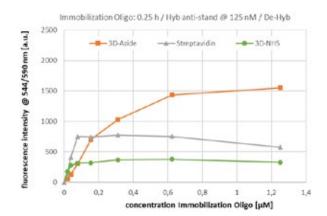
3.4 Comparison of surfaces for oligonucleotide binding

PolyAn's functionalized microplates are particularly suitable for assays that include biomolecules such as oligonucleotides (DNA, RNA, Aptamers) that benefit from oriented coupling and cannot be immobilized efficiently by passive adsorption.

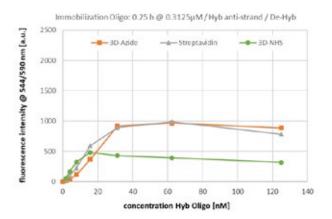
To highlight the advantages and drawbacks of the different surface chemistries offered by PolyAn, we compared some of PolyAn's reactive microplates for a DNA-based hybridization assay.

- 3D-NHS for Amine-terminated oligos:
 Most cost-efficient reactive surface, but low signals due to possible side reactions during immobilization.
- Streptavidin/Neutravidin for biotinylated oligos:
 Fast easy immobilization with best performance at low concentrations, but non-specific binding in complex samples possible.
- 3D-Azide/3D-DBCO for Alkyne-/DBCO-modified oligos:
 Best performance at higher oligo concentrations, but longer incubation times.
- 3D-MTZ plates for TCO-modified oligos:
 The ultra-fast and efficient MTZ-TCO ligation reaction can further improve the results of hybridization assays.

Variation of oligonucleotide concentration



Variation of anti-strand concentration



A 20mer oligonucleotide 5'-modified with either Amino group, Biotin, or DBCO, was immobilized on PolyAn 3D-NHS, Streptavidin-coated, and 3D-Azide microplates, respectively. A corresponding TAMRA-labeled anti-strand was added and the fluorescence intensity of the TAMRA-label was measured as a function of the initial oligonucleotide concentration and the anti-strand concentration.

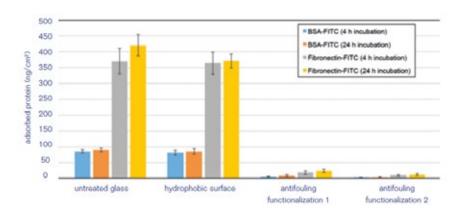
When to select a click chemistry approach:

- For applications in which a maximum signal is required
- For applications where non-specific interactions of the Streptavidin-/Neutravidin-Biotin binding may occur
- For applications in which a covalent, bio-orthogonal binding is required,
 e.g. for PCR or under harsh reaction conditions with organic solvents

4. Antifouling Surfaces and Low Cell Binding Surfaces

PolyAn offers Antifouling surface coatings for a wide range of plastic and glass consumables. Our proprietary coating reduces biofouling and also cell adsorption on nearly any synthetic surface. Products include slides, coverslips, cups, 96-well plates, as well as a wide range of customized products.

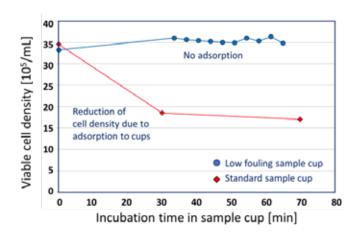
Antifouling Surfaces for Low Protein Binding



PolyAn's antifouling coating is covalently anchored on the base substrate. Therefore, the surface modification is permanent. The autofluorescence and mechanical characteristics of the base substrate are not influenced by PolyAn's surface modification.

Cedex® Sample Cups for Low Cell Binding

The Cedex Low Fouling Sample Cups are functionalized to reduce non-specific binding of cells on the Cup's walls. PolyAn's Low Fouling coating minimizes the cell adsorption, and thus, the potential error when counting the cells. Cedex Low Fouling Cups ensure an increased reliability of cell counting systems when using defined media, different cell lines and variable incubation times.





Key features

- No adsorption of cells to low fouling sample cups
- Minimized standard deviation in counted cell density over time
- Facilitates use of Autosampler for sticky cells/defined media
- Certificate of Analysis for each production batch

Our low cell binding surfaces are also available on other substrates. Please do not hesitate to contact us to discuss your application.

5. Molecular Surface Engineering Service

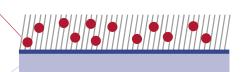
Our Service

Individual Surface Functionalization Solutions
As part of our Molecular Surface Engineering Services, we offer the individual functionalization of substrates for your specific requirements.

Individual solutions

1) Choose your functional surface:

Screen different reactive surfaces to select the optimal matrix for your application



1 1 1

2) Choose your support:

Select the optimal plate format (well shape, material, packaging...) or cartridge design

OR

Bring-your-own plate,...





3) Choose your production scale:

- Transfer from sample size to production scale
- Outsourcing of your production



Custom-made product



Custom product development is the cornerstone capability from which PolyAn's family of products evolved. PolyAn has developed a broad repertoire of manufacturing capabilities that meet customer specifications with regards to tolerances, bio-compatibility, and assay conditions. Our scientists partner with our customers to rapidly build prototypes that enable scaled development and manufacturing.

As a development partner, PolyAn facilitates efficiencies and innovation to maximize your capacities in research and analysis rather than in development and manufacturing. Let us know what you and your company are exploring and we can support you in making that a reality.

6. Ordering Information

We are looking forward to your telephone orders and technical inquiries at our Customer Service and Technical Service Department Monday – Friday. Office hours for telephone inquiries are 9:00 AM to 5:00 PM (Central European Time).

PolyAn GmbH Schkopauer Ring 6 12681 Berlin Germany Tel +49 30 912 078 0 Fax +49 30 912 078 11 Email mail@poly an.de www.poly-an.de

Ordering Process

After placing your order you should receive an order acknowledgment via e-mail within 3 business days. When your products have been shipped, we will notify you via e-mail to provide you with the shipping information, e.g. tracking number.

Shipping and handling

All prices are Ex-Works PolyAn, Berlin. The products can be shipped via FedEx, UPS, DHL Express or airmail. Please provide your account number, if available.



7. Distributors

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