

Functionalized Surfaces for Microarrays





# 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.

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## 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-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 unspecific binding, respectively. As part of our Molecular Surface Engineering services, we offer functionalized consumables for OEM applications, which are tailored to specified customer requirements.





# Index

1.	Product Overview		
2.	Molecular Surface Engineering (MSE)		
3.	. Reactive Surfaces for Immobilization of Biomolecules		
	3.1 Reactive 3D-Surfaces	10	
	3.2 Reactive 2D-Surfaces	15	
4.	Antifouling Surfaces	18	
5.	Substrates and Reagents	19	
	5.1 Planar Substrates	19	
	5.2 Multiwell Plates	20	
	5.3 Buffers & Accessories	21	
6.	3. Molecular Surface Engineering Services		
7.	. Ordering Information		
8.	. Distributors		

## 1. Product Overview

PolyAn is proud to offer one of the broadest portfolios of microarray substrates on the market. Our products include polymer slides and films/sheets, glass slides and coverslips, metal and metal oxide surfaces, as well as functionalized 96-well plates for various microarray applications.

#### Surface Modifications

PolyAn offers customized substrates with a surface modification tailored to your specific application. We offer both 2-dimensional (2D) and 3-dimensional (3D) surfaces with a broad range of reactive groups and functionalities.

#### Glass Slides & Coverslips Polymer Slides 96-Well Plates 2D-Epoxy **3D-Epoxy 3D-Epoxy 3D-Epoxy** 2D-Amine **3D-Amine** 3D-Amine 3D-Amine 2D-Carboxy 3D-Carboxy 3D-Carboxy 3D-Aldehyde 3D-NHS 2D-Aldehyde 3D-Aldehyde 3D-Aldehyde 2D-NHS 3D-NHS 3D-NHS 3D-Azide 2D-PDITC **3D-PDITC 3D-PDITC** 3D-DBCO 2D-Maleimide 3D-Maleimide 3D-Maleimide 3D-Streptavidin 2D-Thiol **3D-Thiol 3D-Thiol 3D-Neutravidin** 2D-Azide 3D-Poly-L-Lysine 3D-Poly-L-Lysine **3D-Antifouling** 2D-Streptavidin 3D-Streptavidin 3D-Streptavidin 2D-Neutravidin 3D-Neutravidin 3D-Neutravidin 2D-Antifouling **3D-Antifouling 3D-Antifouling**

PolyAn also functionalizes thin plastic films, membranes, metal and metal oxide surfaces (wafers), as well as polymer particles with our 3D-reactive matrices.

#### Accessories & Reagents

PolyAn offers blocking and washing buffers for our surfaces to facilitate your lab work. As part of our technical service we can provide standard handling protocols for our surfaces and are also happy to personally answer questions or discuss a customized solutions for your application.

PolyAn is the European distributor of Grace Bio-Labs' Nitrocellulose film slides. We are offering a broad selection of superstructures, incubation chambers, and other accessories to make lab work with slides & coverslips easier.

# 2. Molecular Surface Engineering (MSE)

#### 2D- and 3D-reactive Surfaces

PolyAn's substrates can be functionalized either with a 2-dimensional (2D) surface or with a 3-dimensional (3D) surface. Both surface types are covalently anchored on the base substrate. Our MSE technology gently binds the functional layer onto the surface without damaging the base substrate or altering its optical properties. Our broad range of different surface morphologies, reactive groups, and substrates enables the selection of an optimal combination for each specific application.



<u>2D-Slide with functional groups</u> e.g. Epoxy

- Ultra-thin (mono)layer
- Rigid structure
- Cost-effective reactive surface
- Suitable for glass, metal, and metal oxide surfaces



<u>3D-Slide with Antifouling Matrix</u> and functional groups e.g. Epoxy

- Thickness: 1-50 nm, depending on application
- Swellable hydrogel
- Tentacular branched polymer structure
- Partly cross-linked, but fully penetrable for small molecules





PolyAn Antifouling Matrix

- Adjustable contact angle
- Variable density / surface
- concentration of functional groups — Co-functionalized with Antifouling
  - Matrix

## 3D-Surfaces for High-Performance Applications

PolyAn's high performance surfaces (e.g. Microarray Slides) are functionalized with a 3D-Surface chemistry comprised of a long chain polymer containing a homogeneous density of reactive functional groups. This 3D-reactive Surfaces can then be used for the covalent attachment of biomolecules such as proteins, peptides, and oligonucleotides:



## **Tunable Surface Properties**

The morphology of the functional surface, and thus, the number of the reactive groups can be fine tuned within a narrow range. This yields a number of advantages:

Low fluorescence background	Covalent binding of functional layer on the substrate without increasing the autofluorescence
Low non-specific binding	Combination of reactive functional groups with PolyAn antifouling matrix
Optimal density and high accessibility of functional groups	Morphology and thickness of functional layer tailored to the desired application
Uniform spot morphology	Narrow variation of surface properties e.g. contact angle, homogeneous distribution of functional groups
Wettability	Tunable surface hydrophilicity / hydrophobicity (contact angle)



The contact angle is a measure of the wettability (hydrophilicity) of the surface. A narrow specification of the contact angle ensures uniform spot morphology (within a slide and from batch-to-batch) and can also be used as an indicator for the overall homogeneity of a surface.

#### **Excellent Shelf Life**

PolyAn's slides are characterized by a long shelf-life when stored dry, at room temperature and protected from sunlight. All slides are packaged in boxes under Argon atmosphere to avoid contamination with particles. The Argon atmosphere also minimizes degradation of the reactive surface through contact with air or humidity. Our slides are available in boxes of 5 and 25 slides, respectively.

#### Surface Homogeneity

PolyAn's functionalized slides are characterized by the narrow variation of their surface properties, e.g. contact angle and loading, as illustrated in the adjacent figures. The integrated antifouling matrix significantly reduces the background fluorescence of the peptide arrays used in this experiment.



The image above nicely illustrates the low spot-to-spot variation and excellent spot morphology of PolyAn's reactive 3D-matrix. Additionally, the integrated antifouling matrix ensures a low background outside of the spots resulting in an excellent signal-to-noise ratio.

#### Handling & Buffers

The functionalized substrates should be used in a dust-free environment. Particles on the slide surface may cause defects in the probe binding and cause uneven background.

Unreacted biomolecules and buffer residues must be removed from the slide surface after printing by extensive washing. Additionally, it is necessary to rigorously de-activate remaining free reactive groups on the slide. In order to ensure an optimal performance we advise to use small blocking molecules for de-activating any reactive groups hidden in the 3D-matrix to achieve an optimal performance. PolyAn offers a range of buffers & blocking reagents that are optimized for our reactive surfaces.

Please do not hesitate to contact us, if you have any questions regarding the handling of our surfaces. As part of our technical service we will be happy to support your work.

## 3. Reactive Surfaces for Immobilization of Biomolecules

#### Immobilization of biochemical Species onto Surfaces

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.



Support, e.g. Glass, Plastic, Metal

The strongest immobilization method in biochemistry is the covalent attachment. A covalent bond is formed by sharing of electrons between two atoms. The dissociation energy for a typical covalent bond is 420 kJ/mol, and thus, far higher compared to the 130 kJ/mol of a typical electrostatic interaction. It can be distinguished between a covalent attachment of activated targets and a covalent attachment of biological species on activated surfaces.

# Overview of reactive Groups and Functionalities

Functional Group	Structure	Application examples	
Ероху	°	For binding of nucleophiles such as Amine-, Thiol-, and Hydroxy-containing molecules	
Amine	NH <sub>2</sub>	For reaction with activated Carboxy groups, or electrostatic adsorption of negatively charged species	
Carboxy	Он	For EDC/NHS-mediated reaction with Amine groups, or electrostatic adsorption of positively charged species	
Aldehyde	→ H	For reaction with Amine- and Hydrazine-containing molecules	
NHS		For direct binding of Amine-containing molecules	
PDITC	-H H - N C s	For binding of nucleophiles such as Amine-, Thiol-, and Hydroxy-containing molecules	
Maleimide		For direct binding of Thiol-containing molecules	
Thiol	—SH	For reaction with Maleimides, Thiol-containing molecules, and Alkenes (Thiol-ene reaction)	
Azide	NN NN	For reaction with Alkyne groups and DBCO-modified molecules via Click Chemistry	
Alkene		For binding of Thiol-containing molecules and Polymerization reactions	
Poly-L-Lysine	$\begin{array}{c} OH \\ H \\ NH_2 \end{array} \\ H \\$	For reaction with activated Carboxy groups, or electrostatic adsorption of negatively charged species	
Streptavin/Neutravidin	×	For coupling of Biotin-functionalized molecules	

#### 3.1 Reactive 3D-Surfaces

**Epoxy Surfaces** 



for covalent coupling of nucleophiles such as Amine-, Thiol-,

Epoxides are cyclic ethers with a highly strained three-membered ring. The Epoxy rings can be easily reacted with nucleophiles e.g. Amines, Hydrazines, Thiols, Hydroxides, and Carboxyl groups under acidic or basic conditions. Epoxy surfaces have a longer shelf-life compared to NHS-esters and 1,4-Phenylene isothiocyates (PDITC), as they are less susceptible to humidity and are stable at temperatures of up to 40°C.

#### Amine and Carboxy Surfaces for Adsorptive Immobilization

for non-covalent coupling of charged biochemical species via ionic interaction.



An adsorptive immobilization is a non-covalent coupling method on solid supports which is realized by electrostatic (Coulombic and/or Van-der-Waals) interactions, hydrogen bonds, and hydrophobic interactions of the reactants. The dissociation energy for typical electrostatic bonds is 130 kJ/mol, which is about a third of the strength of an average covalent bond. In order to achieve an optimal adhesion the probe buffer and the adhesion conditions (pH-value) have to be optimized. For proteins with different surface charges, also an oriented immobilization is possible.

# Amine Surfaces

for covalent binding of Oligonucleotides via UV-crosslinking.



Amine surfaces can be used to immobilize e.g. DNA. The oligonucleotide is bound electrostatically on the 3D-Amine surface with its negatively charged backbone or its 5' phosphate group. For immobilization of nucleic acids we recommend an UV-crosslinking after adsorption: During the UV irradiation the base Thymine forms radicals which undergo a Hydrogen-abstraction to form a covalent bond with the 3D-Surface.

#### **PDITC Surfaces**

for covalent coupling of nucleophiles such as Amine-, Thiol-, and Hydroxy-containing molecules.



PDITC (1,4-Phenylendiisothiocyanate) is a homobifunctional crosslinker, that immediately reacts with nucleophiles e.g. Amines, Hydrazines, Thiols, and Hydroxides to form stable covalent bonds. After attachment of the biochemical species the surfaces must be blocked with a blocking buffer containing small molecules that can access all reactive groups within the 3D-Matrix.

#### Poly-L-Lysine Surfaces

with a high surface group density of both primary and secondary amines.



Covalently attached Poly-L-Lysine offers a high density of surface amines. Slides coated with Poly-L-Lysine can be used as adhesive microscope slides for the electrostatic coupling of biomolecules or biological samples, e.g. DNA, cells, tissues.

#### **Carboxy Surfaces**

for EDC/NHS-mediated coupling of Amine-containing molecules.



Carboxy groups can be activated with EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide) and NHS (N-hydroxysuccinimide) to form a highly reactive intermediate. This intermediate can be easily reacted with the  $NH_2$ -groups of biochemical species, such as Amines and Hydrazines.

#### **NHS** Surfaces

for direct binding of Amine-containing molecules.



NHS-esters react immediately with molecules containing  $NH_2$ -groups, such as Amines and Hydrazines, to form a covalent bond. However, due to its high reactivity the NHS-ester is susceptible to hydrolysis. Thus, NHS-activated surfaces should be processed promptly after opening the sealed bags.

#### Aldehyde Surfaces

for covalent coupling of Amine-containing molecules.



Aldehyde groups bind to Amines, Hydrazines, and Aminoalkoxyacetyl-containing molecules. In an intermediate state the Aldehydes form an Imine-group with the Amines (Schiff-base). In order to increase the bond strength it is also possible to reduce the Imines with e.g.  $NaBH_4$  or TCEP (Tris(2-carboxyethyl)phosphine) to form stable Amines.

**Thiol Surfaces** 

for covalent coupling of Thiol-containing biomolecules and for the Thiol-ene reaction with Alkenes.



Thiol surfaces react with other Thiol-containing probes in an oxidative coupling reaction to form so-called "disulfide bridges". This reaction can be achieved with mild oxidizing agents such as peroxides, iodine or bromine, and even atmospheric oxygen.

Thiol surfaces can also be applied to covalently bind alkene moieties with high yield and stereoselectivity in a Click chemistry reaction (not shown). This so-called thiol-ene reaction proceeds either through a Michael addition when catalyzed by a base or nucleophile, or via a free-radical addition when initiated by light, heat, or a radical initiator.

#### Maleimide Surfaces

for direct binding of Thiol-containing molecules.



Maleimide-esters react immediately with Thiol-groups of biochemical species. The Thiol-groups can be either natively present in the (bio)molecule, e.g. through the amino acid cysteine in proteins, produced via reductive cleavage of disulfide bonds with a reducing agent such as Dithiothreitol (DTT, Cleland's Reagent), or selectively introduced e.g. with 2-Iminothiolane (Traut's reagent) for amine-containing molecules. Similar to NHS-esters, Maleimide surfaces are susceptible to hydrolysis, and thus, should be processed promptly after opening the sealed bags.





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  $K_p = 10^{-15}$  mol/L. Thus, it is often applied in bioanalytical applications.

Streptavidin is a non-glycosylated protein purified from the bacterium Streptomyces avidinii. Neutravidin is a deglycosylated form of the native Avidin protein from egg white. Both Biotin-binding proteins can be distinguished by their isoelectric point, specificity, and non-specific binding:

	Avidin	Streptavidin	Neutravidin
Molecular Weight	67 kDa	53 kDa	60 kDa
Biotin-binding Sites	4	4	4
Isoelectric Point (pl)	10	6.8 – 7.5	6.3
Specificity	Low	High	Highest
Affinity for Biotin ( $K_D$ )	10 <sup>-15</sup> mol/L	10 <sup>-15</sup> mol/L	10 <sup>-15</sup> mol/L
Non-specific Binding	High	Low	Lowest

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

#### 3.2 Reactive 2D-Surfaces

For cost-sensitive applications PolyAn has developed a range of 2-dimensional (2D)-reactive glass slides and coverslips that are manufactured from high quality glass with an ultra-flat surface and low inherent fluorescence. The glass is coated with a thin layer that will covalently bind most types of biomolecules. The defect-free surface features uniform functional layers that provide a high covalent coupling efficiency together with a very low background. The slides and coverslips are easy to use, and are fully compatible with all commercially available arraying and scanning instruments as well as fluorescence microscopes.

Besides standard glass slides and coverslips, PolyAn also offers the functionalization of metal and metal oxids. Upon request the density of the functional groups and the contact angle can be optimized for your application.

#### 2D-Epoxy

for covalent coupling of nucleophiles such as Amine-, Thiol-, and Hydroxy-containing molecules.



#### 2D-Amine / 2D-PDITC

for covalent coupling of nucleophiles such as Amine-, Thiol-, and Hydroxy-containing molecules.



#### 2D-Carboxy / 2D-NHS

for direct binding of Amine-containing molecules.



## 2D-Aldehyde

for covalent coupling of Amine- and Hydrazine-containing molecules.



#### 2D-Maleimide

for direct binding of Thiol-containing molecules.



#### 2D-Thiol

for reaction with Maleimides, Thiol-containing molecules, and Alkenes (Thiol-ene reaction).



# 2D-Azide

for reaction with Alkyne groups and DBCO-modified molecules via Click Chemistry.



## 2D-Alkene

for reaction with Thiol-containing molecules (Thiol-ene reaction) and Polymerization reactions



# 4. Antifouling Surfaces

#### Reduction of non-specific Binding

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



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



## Increased Wettability of Microfluidic Structures

Degree of surface functionalization



Retarded flow velocity of ink in non-modified channels Enhanced flow velocity of ink in 3D-hydrophilized channels

## 5. Substrates and Reagents

#### 5.1 Planar Substrates

#### <u>Glass</u>

Glass can be functionalized with all of the 2D- and 3D-reactive surfaces from PolyAn's portfolio that can be found in this brochure.

The most commonly used glass sizes are listed in the table below:

	Size	Thickness	Packaging
Glass Slides	25 mm x 75.6 mm	1 mm	5 slides/pack or 25 slides/pack
Glass Sheets	74 mm x 110 mm	1 mm	5 sheets/pack
Coverslips	25 mm x 60 mm	0.17 mm	5 coverslips/pack

Other dimensions and thicknesses are available upon request. PolyAn also offers the modification of wafers as well as metal- and metal oxide-coated substrates.

#### **Polymers**

Polymers are only available with our 3D-reactive surfaces. Standard materials are cyclic olefin polymer (COP) and polymethyl methacrylate (PMMA) which are both suitable for applications which require a low autofluorescence and excellent optical properties.

An overview of the standard sizes and materials is listed below:

	Size	Thickness	Packaging
COP slides	25 mm x 75.6 mm	1 mm	5 slides/pack or 25 slides/pack
COP film	25 mm x 75 mm	0.188 mm	5 slides/pack or 25 slides/pack
COP film	74 mm x 110 mm	0.188 mm	5 sheets/pack
PMMA slides	25 mm x 75 mm	1 mm	5 slides/pack or 25 slides/pack
PMMA sheets	74 mm x 110 mm	1 mm	5 sheets/pack

Additionally, also other polymers, e.g. PP, PE, PS, or PVDF, can be equipped with reactive as well as low binding surfaces. As part of our Molecular Surface Engineering services we offer the customized functionalization of plastic substrates.



<u>Pre-scored sheets for cartridges & microfluidics</u> Functionalized COP films with low autofluorescence that can be easily cut into suitable formats post printing. The finished film-chip can be easily integrated into cartridges or microfluidic devices.

#### 5.2 Multiwell Plates

#### Multipart 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
- 0.17 mm coverslip glass
- (Type #1.5, #1.5H, ...)
- 1 mm PMMA

#### <u>Surfaces</u>

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





#### 96-Well Plates

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

Plate designs:

- 96-well white, 12 x 8-strip, flat bottom
- 96-well transparent, 12 x 8-strip, flat bottom
- 96-well µClear® microplate, PS, black

Other plate designs are available upon request.

#### Surfaces:

- 3D-Epoxy, 3D-NHS, 3D-Aldehyde, 3D-Azide
- Streptavidin and Neutravidin

#### 5.3 Buffers & Accessories



PolyAn provides washing and blocking buffers that are optimized for PolyAn's 2D- and 3D-reactive surfaces. The PolyAn buffers promotes highly efficient coupling of biomolecules and increases the signal-to-noise ratio by minimizing unspecific binding.

Buffer / Solution	Used for	Ingredients
PolyAn Blocking A	Surface passivation of NHS, Epoxy, Aldehyde and PDITC modifications	Anionic surfactants, Amines, pH=9.0
PolyAn Blocking B	Surface passivation of Streptavidin, Amines, PDITC modifications	Proteins, Surfactants
Wash solution I + II (suited for DNA microarrays)	Optimal wetting and reduction of non-specific interactions	I. lonic surfactants, Salts II. Lower concentrated ionic surfactants, Salts
Wash solution III (suited for DNA microarrays)	Optimal wetting and reduction of non-specific interactions	Lower concentrated salts



#### <u>Accessories</u>

PolyAn offers a range of useful accessories to facilitate handling of glass slides, coverslips, and glass plates.

Seals and multi-well chambers are available for a variety of applications such as carrying out post-printing binding reactions of microarrays, immunohistochemistry, simple incubation, perfusion, imaging, hybridization, microfluidics, and tissue/cell staining microscopy.

Numerous conformations for well shape, size and depth along with several materials such as silicone and polycarbonate are available.

Please contact us for assistance in selecting the correct product for your application or to discuss a custom device.

# 6. 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 specific requirements.

#### Individual solutions

# 1) Choose your functional surface:

- 2D- / 3D-reactive surfaces
- Biomolecule coatings
- Adsorptive surfaces
- Antifouling surfaces
- Low Cell Adhesion
- Customized hydrophilicity



#### 2) Choose your support:

<u>Glass slides, coverslips</u> Various thicknesses and dimensions possible

Plastic support materials e.g. PE, PP, PES, PS, PVDF, PMMA, COC and COP

#### Plates, Cartridges

<u>Coating with metal and metal oxides</u> e.g. Gold, Silver, Platinium, ITO, Tantal oxide,...



#### 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.

# 7. Ordering Information

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

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## **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.



# 8. Distributors

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