Stratospheres for Peptide Synthesis

The StratoSpheres product line encompasses a wide range of polymer resin supports for the development and large scale manufacture of peptide APIs. These include resins for Boc and Fmoc chemistries and include CMS, AMS, Rink, Wang and AmphiSpheres. StratoSpheres are high performance, high quality resins that enable you to make the highest quality APIs. They will decrease your time to market and accelerate the manufacturing process. Our large scale resin manufacturing facility means that we have your needs covered from early development, through clinical trials to successful market launch, while providing you with long term security of supply.

Copolymerization is used to produce StratoSpheres CMS polymer, which is the precursor to many of our other resins. This provides superior synthesis performance, and therefore better quality and higher purity peptides. ISO 9001 manufacturing processes are designed to produce high quality resins, with tight particle size distribution, and high batch to batch reproducibility. Multi kg batch sizes means we have the capacity to supply metric ton quantities of resin per year! Our willingness to partner with you to ensure the success of your project and provide you with the long term security of supply. This can include, custom manufacture, customizing specifications, scale-up to ensure economy of scale, support for Quality agreements and audits, as well as on time delivery anywhere in the world.

Particle Technologies

Product Types

Quality
Our manufacturing techniques, particularly the use of copolymerization, provide the highest quality supports. Agilent is ISO 9001:2008 accredited, and we regularly entertain customer audits and quality inspections.

Capabilities
Agilent has a purpose-built production facility located in the UK. Particles are produced by copolymerization in multi-kilogram quantities with batches typically up to 100 kg in size. Chemical modification (attachment of appropriate handles, linkers and functional groups) is carried out in our kilolab facilities (20 L glass vessels) from 100 g to 2 kg. Larger production batches are produced in 50 L, 200 L or 500 L glass lined or hastelloy vessels in batches from 3 kg to 80 kg. Our annual capacity is currently ~ 2 tonnes.
**Particle Technologies**

**Product Types**

**What are StratoSpheres?**
The StratoSpheres product line encompasses a wide variety of peptide synthesis resins. These products are specifically designed to provide enabling tools for organic chemists, particularly in the field of high throughput chemistry and drug design and development. The StratoSpheres range is synonymous with quality, at an affordable price.

**Why use Polymer Beads?**
Since the inception of solid phase synthesis by Bruce Merrifield in 1963, filtration of polymeric particles has proven to be much more efficient than many traditional work-up procedures such as liquid-liquid extraction, re-crystallization or chromatography.

**Microporous Particles (1% DVB)**
Microporous is the term used to describe very lightly crosslinked polystyrene beads. When dry, the beads are hard and spherical, however most of the functionality is contained within the interior of the particles. In order to gain access to the functional interior, it is necessary to swell the beads. In effect the polymer chains try to dissolve, but the light crosslinking ensures that the material remains in gel form. Once swollen, reagents can readily diffuse into the interior of the beads, and excess reagents or by-products can be washed away.

1% DVB (divinylbenzene) is sufficient to lightly crosslink polystyrene particles. When swollen in solvent, the particles will almost double in diameter (resulting in a six to eight fold increase in volume). Using a poor solvent will cause swollen beads to shrink and inhibit diffusion, meaning washing procedures frequently involve a selection of solvents which will swell and then shrink the beads. If the beads are to be dried, they should be washed in a solvent which causes them to reduce in size.

Agilent also manufactures a range of Macroporous resins for scavenger as well as for Solid Phase Extraction (SPE) applications. Please contact your local Agilent office for more information.

![Composition of a microporous particle](image-url)
Particle Technologies

**Product Types**

**What is Copolymerization?**

Agilent’s expertise is using copolymerization to introduce functional groups into the polymer beads. This is a superior approach to the traditional method of taking a polystyrene particle and trying to functionalize it; the level of functional groups (which form the loading of the material) is more readily controlled. Consequently the reproducibility of StratoSpheres particles is exceptional and the unwanted introduction of by-products can be avoided.

![Chemical structures](image)

**Reaction Rates**

There are many misconceptions about reaction rates when using polymer supports. Many people believe that reactions are always slower on a polymer than in solution, this is not necessarily so. As with most chemical reactions, the rate is governed primarily by the concentration. With a 1.0 mmol/g microporous resin, 1 g may be swollen in 10 mL of solvent. The effective concentration is therefore 0.1M. Using an excess of reagents will help to force reactions to completion. Many of our products have much higher loading and therefore are more concentrated.

**Particle Size**

A wide range of particle sizes can be produced using suspension polymerization techniques. The most common sizes are 75-150 µm (100-200 mesh) for solid phase synthesis applications and 150-300 µm (50-100 mesh) for solution phase applications. Smaller or larger bead sizes are also available, however, during the manufacturing process it is very important to remove any undersized particles, or ‘fines’, which can block filters and frits, and inhibit drainage. The larger beads are somewhat easier to handle and can be readily dispensed using manual, semi-automated or automated techniques commonly employed in high throughput synthesis. The smaller bead sizes are more appropriate for repeated swell-shrink cycles that are encountered in solid phase peptide synthesis. Custom bead sizes are available upon request.

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<tr>
<td>400 - 500 µm</td>
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**Hydrophobicity**

Polystyrene is the most commonly used material for resins and supports. It is particularly easy to handle because it forms glassy beads when dry, and will swell readily in appropriate solvents. The choice of solvent to swell a microporous polystyrene is somewhat limited – the polystyrene backbone is hydrophobic and therefore requires the use of tetrahydrofuran, dichloromethane, toluene and other non-polar solvents. More polar solvents that may be used include dimethylformamide, dimethylacetamide and N-methylpyrrolidone. Other common organic solvents, such as methanol, acetonitrile and diethyl ether do not swell polystyrene, and are therefore unsuitable as reaction solvents. The exception to this is macroporous polystyrene, where the functionality is contained within the pores of the bead on the internal surface area and can be accessed using a much wider choice of solvents.

Using proprietary copolymerization techniques gives StratoSpheres particles the highest level of reproducibility and exceptional quality.
Resin Loading

Using proprietary copolymerization techniques, the level of functionality in a polymer can be readily controlled without the problems that other methods of functionalization can bring. The materials obtained by copolymerization show much greater reproducibility and are free from by-products arising from side reactions.

As has already been shown, the resin loading (and its swell) determines the concentration of reactive sites. For scavenger resins and supported reagents it is desirable to have a high level of functionality. For synthesis supports the level of loading is generally somewhat less. Using a 1.0 mmol/g loading resin to produce a typical peptide, after about 8-10 amino acids have been coupled together, 1 g of peptide per gram of resin will be left.

The peptide-resin assembly will begin to behave more like a peptide than polystyrene. If you wish to make a large peptide (20–30 amino acids), it will almost certainly be necessary to start with a resin with a lower loading. Agilent therefore offers a wide choice of resin loadings suitable for peptide synthesis.

For small molecule synthesis, drug-like molecules have much lower molecular weights than peptides. The loading of the resin used to synthesize such a compound can therefore be much higher.

For scavenger resins we have prepared polystyrene supports where nearly every other polystyrene ring is substituted for maximum effect. Even our macroporous materials are produced using copolymerization in order to maximize the loading.

Microwave Compatibility

The use of microwave reactors to speed up chemical reactions is a concept which has been widely adopted in high throughput chemistry applications. In many cases, the reaction is performed at elevated temperatures for very short periods of time, much shorter than the time required for reaction when using traditional heating techniques. This has lead to the question of whether polymer supported reagents and catalysts may be used in microwave assisted organic synthesis. The answer is not always straightforward. In most cases, polystyrene-based materials are stable to very high temperatures, particularly for short exposure times.

Polystyrene is thermally stable in excess of 250 °C. However, it must be remembered that under some reaction conditions, it is possible to react with the aromatic rings of polystyrene themselves, and any functional groups on the polymer may be subject to thermal degradation or side-reactions also.

It is particularly important to consider the temperature of the reaction and the solvent being used. In some cases, the polymer will swell to a greater extent under microwave conditions and may therefore occupy a larger volume or imbibe a larger proportion of solvent. It is also necessary to consider where the microwave energy is being absorbed. Although it is possible to run microwave assisted reactions at elevated temperatures in solvents such as dichloromethane, for example, how is the temperature of the reaction being raised? Is the microwave energy being absorbed primarily by a polar functional group on the polymer and then heat being transferred to the solvent? If this is the case, then microwave heating of the polymer is occurring and damage could ensue.

It is preferable to use a solvent which will absorb most of the microwave energy directly, or at least to use a solvent mixture so that the energy is absorbed more generally. Solvents such as dimethylformamide or N-methylprollidone are preferable to dichloromethane. Methanol is also a good solvent for use in a microwave.

The reproducibility and reliability of StratoSpheres particles are essential for polymer-assisted synthesis, whether using microwave or conventional heating.
How to use this section

The product section is an alphabetical listing of all available products. If you already know the product name you can quickly find the relevant information on that material: specifications, up-to-date bibliographic references for applications and ordering information.

Product Name

Description:
Fmoc Rink amide AMS resin

Application:
Solid Phase Peptide Synthesis, Synthesis of Amides

Rink amide resins are often the support of choice for solid phase synthesis of peptide amides using Fmoc chemistry. Prior to use, PL-Rink requires the removal of the Fmoc protecting group, which can be accomplished using standard deprotection protocols, e.g., 25% piperidine in DMF for 30 min, followed by thorough washing prior to use. This resin is very versatile, as the initial amino acid can be attached using any conventional amide bond forming chemistry (peptidyl chloroformyla-ture esters only). This coupling reaction can also be monitored using colorimetric tests such as the Kaiser test. Following assembly of the protected peptide sequence, the N-terminal Fmoc protection is removed. At the same time, any tert-butyl based side chain protection is removed by cleavage of the peptide amide from the resin using 95% TFA solution.

References

Ordering Information

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</table>

Additional Information
PL-Rink is sold under license from Aventis.
Agilent manufactures in multi kg quantities. Please enquire for details.
AmphiSpheres

Description: Polystyrene, PS, Polyethyleneglycol, PEG
Application: Solid Phase Peptide Synthesis

Specifically designed for solid phase peptide synthesis, AmphiSpheres amphipathic resin is a key product in the StratoSpheres product family.

As the name suggests, this type of material contains both hydrophobic (polystyrene, PS) and hydrophilic (polyethyleneglycol, PEG) components. This subtly changes the swell characteristics of the material allowing a broader range of solvents to be used. At the same time, the active functionality is located at the end of a PEG chain which helps promote reactivity.

Two versions are available with differing PEG content:

• AmphiSpheres 20 has 20% w/w PEG content and a loading of 0.7 mmol/g.
• AmphiSpheres 40 has 40% w/w PEG content and a loading of 0.4 mmol/g.

AmphiSpheres 20 contains 20% w/w polyethylene glycol and therefore retains a high loading per gram and has handling characteristics close to that of “glassy” polystyrene. This means that the yield of product is not compromised to the same degree as with larger PEG chains.

AmphiSpheres 40 contains 40% w/w polyethylene glycol and uses a longer PEG chain than AmphiSpheres 20. The amount of PEG is noticeable in that the material is more difficult to shrink down without becoming sticky. However the increased length of PEG chain can give significantly improved results in the synthesis of “difficult” peptide sequences.

Attachment of the appropriate linker or handle enables the material to be used for the synthesis of peptide acids and peptide amides.

Ordering Information

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Additional Information

Agilent manufactures in multi kg quantities. Please enquire for details.
Aminomethylstyrene resin is a particularly versatile material suitable for the attachment of a variety of spacers, handles and linkers (for use in solid phase synthesis).

A number of methods exist for preparing aminomethylstyrene, usually by direct aminomethylation of polystyrene or conversion of chloromethyl functionalized particles. PL-AMS is prepared by the latter approach as this enables the copolymerized PL-CMS to be used as a starting material. It is therefore possible for a very wide range of loadings and particle size combinations to be prepared to suit any given application.

The preferred method of attachment of linkers or handles is through an amide bond. We prepare a number of products in this way, including PL-Rink.

**References**

## PL-AMS Resin

### Ordering Information

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### References continued


### Additional Information

Agilent manufactures in multi kg quantities. Please enquire for details.
PL Cl-Trt-Cl Resin

**Description:** 2-Chlorotritylchloride Resin

**Application:** Solid Phase Peptide Synthesis, Synthesis of Protected Peptide Fragments

PL Cl-Trt-Cl is a very popular support for peptide synthesis using the Fmoc approach. It benefits from ease of attachment of the first amino acid residue and was originally designed as a particularly acid-sensitive resin for the production of fully protected peptide fragments. In this mode, cleavage of peptides can be accomplished by very mild acidolysis, leaving any tert-butyl side chain protection intact. This enables the isolation and subsequent assembly of protected fragments to produce much larger peptides.

This resin can be used for the synthesis of a wide range of organic acids, and has also been used for the synthesis of amines, phenols and other alcohols. For example, attachment of Fmoc-hydroxylamine to the resin, followed by coupling of an organic acid to the deprotected hydroxylamine resin, will allow hydroxamic acids to be created upon cleavage.

![Chemical Structure](image)

References


Ordering Information

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Additional Information

Agilent manufactures in multi kg quantities. Please enquire for details.
**PL-CMS Resin**

**Description:** Chloromethylpolystyrene; poly(styrene-co-chloromethylstyrene)

**Application:** Acid Labile, Synthesis of Acids

Commonly known as Merrifield resin, PL-CMS is a copolymer support designed for solid phase synthesis of peptides using Boc chemistry. Boc-amino acids are typically attached to the resin as a cesium salt, although other techniques have also been used. A slight excess of acid is neutralized with cesium carbonate and the activated acid isolated by evaporation. A solution of the activated acid in DMF should be reacted with DMF-swollen PL-CMS at an elevated temperature (e.g., 50 °C) overnight. Cleavage typically requires treatment with very strong acid such as HF or TFMSA.

Other useful techniques for cleavage include saponification or hydrolysis to create free acids, transesterification to create methyl esters, or aminolysis to form carboxamides.

PL-CMS can be used to generate a variety of other supports by the attachment of appropriate linkers, particularly through Williamson ether synthesis.

Note: specialist equipment and training is required to safely perform HF cleavage operations.

This plot shows the exceptional loading reproducibility of ten batches of PL-CMS 1.0 mmol/g, 75-150 µm manufactured over a period of more than a decade.
PL-CMS Resin

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References


Additional Information

Agilent manufactures in multi kg quantities. Please enquire for details.
PL-MBHA Resin

Description: 4-Methylbenzhydrylamine resin
Application: Solid Phase Peptide Synthesis, Synthesis of Amides

Although PL-MBHA is traditionally used as a support for solid phase synthesis of peptide amides using Boc chemistry, the acid stability of the resin enables other molecules to be produced. These include molecules such as β-lactam derived compounds, where reagents and by-products from the process might otherwise cause premature cleavage from the resin.

Initial amino acids and other carboxylic acids can be coupled directly to the resin using standard coupling techniques.

Cleavage typically requires treatment with very strong acid such as HF or TFMSA.

Sometimes Fmoc peptide synthesis is performed on PL-MBHA to allow removal of side chain protecting groups while the peptide chain remains attached to the resin. HF cleavage then ensures that only the desired unprotected peptide amide is released into solution, free from contamination with cleaved side chain protecting groups.

PL-MBHA can also be used for attachment of linkers in a similar manner to PL-AMS resins.

Note: specialist equipment and training is required to safely perform HF cleavage operations.

References
(13) Bishop, B. M.; McCafferty, D. G.; Erickson, B. W. Tetrahedron 2000, 56, 4629-4638.

Ordering Information
PL-MBHA Resin (1% DVB) 1 kg
1.1 mmol/g, 75-150 μm PL3484-6799

Additional Information
Agilent manufactures in multi kg quantities. Please enquire for details.
PL-PEGA Resin

Description: Acryloylated O,O'-bis(2-aminopropyl)polyethylene glycol resin
Application: Solid Phase Synthesis, Enzyme Permeation

PL-PEGA is a unique material designed to provide a complete hydrophilic support, capable of allowing permeation by enzymes. This means that solid phase synthesis can be used to generate compounds which remain attached to the beads, and can be used directly in a variety of assays. PL-PEGA has been widely used in both peptide synthesis and the solid phase synthesis of oligosaccharides. The presence of a primary amine functionality means it is possible to attach a wide variety of common handles or linkers using standard coupling chemistry.

PL-PEGA 0.4 mmol/g, 150-300 µm (wet bead size) is manufactured using a commercially available PEG compound of around 800 MW. The molecular weight cut-off for this resin is around 35 kD. Swell is approximately 8-12 mL/g in DMF, 11-15 mL/g in MeOH and 15-25 mL/g in water.

PL-PEGA 0.2 mmol/g, 300-500 µm (wet bead size) is manufactured using a commercially available PEG compound of around 1900 MW. The molecular weight cut-off for this resin is around 70 kD. Swell is approximately 15-25 mL/g in DMF, 15-25 mL/g in MeOH and >30 mL/g in water.

On-bead screening
PL-PEGA Resin

References

Ordering Information

PL-PEGA Resin

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PL1432-4679

Additional Information
PL-PEGA is sold under license from Carlsberg Laboratory.
The resin is supplied in a swollen form and the quantity supplied corresponds to the equivalent dry weight of the resin.
However, this resin should not be dried.

www.agilent.com/chem/stratospheres
PL-Rink Resin

**Description:** Fmoc Rink amide AMS resin

**Application:** Solid Phase Peptide Synthesis, Synthesis of Amides

Rink amide resins are often the support of choice for solid phase synthesis of peptide amides using Fmoc chemistry.

Prior to use, PL-Rink requires the removal of the Fmoc protecting group, which can be accomplished using standard deprotection protocols, e.g., 20% piperidine in DMF for 30 min, followed by thorough washing prior to use.

This resin is very versatile, as the initial amino acid can be attached using any conventional amide bond forming chemistries (symmetrical anhydrides, active esters, etc.). This coupling reaction can also be monitored using colorimetric tests such as the Kaiser test.

Following assembly of the protected peptide sequence, the N-terminal Fmoc protection is removed. At the same time, any tert-butyl based side chain protection is removed by cleavage of the peptide amide from the resin using 95% TFA solution.

**References**

**Ordering Information**

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**Additional Information**

PL-Rink is sold under license from Aventis. Agilent manufactures in multi kg quantities. Please enquire for details.
**PL-Wang Resin**

**Description:** 4-Hydroxymethylphenoxymethyl polystyrene

**Application:** Solid Phase Peptide Synthesis, Synthesis of Carboxylic Acids

PL-Wang is a 4-alkoxybenzylalcohol functionalized polystyrene, prepared from copolymerized PL-CMS. This support was originally designed for solid phase peptide synthesis using Fmoc protection strategies and is cleaved using ~95% TFA. It is also particularly useful for solid phase synthesis of small molecules which have a carboxylic acid functional group. Amino acids and carboxylic acids are attached to this resin through esterification. Care should be taken during the activation procedure to minimize any risk of racemization.

Acidic alcohols, particularly phenols, may also be attached to PL-Wang.

Wang resins have also been converted to carbamate functionalized materials in order to prepare substituted amines.

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**References**

PL-Wang Resin

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Additional Information

Agilent manufactures in multi kg quantities. Please enquire for details.
# Index

**Products by Product Name**

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Agilent chemistries: providing you confidence and control

Agilent is ISO 9001:2008 accredited, with over 35 years’ experience in bead manufacture and applications development. Agilent’s technologies are widely used in chromatography, life science and pharmaceutical chemistries. We manufacture superior quality, reliable particles for bead-based assays, chromatography media, supports for peptide and oligonucleotide synthesis, and resins for high throughput chemistry. All Agilent’s beads are manufactured under stringent quality controls to ensure batch-to-batch reproducibility of physical and chemical properties.

You can be confident that Agilent’s meticulous end-to-end oversight of production delivers you the highest consistency and performance. With more than 40 years of experience in the production of polymer chemistries, our team is committed to continuous development so that you stay ahead of the curve with the technology which will make you the most productive.

You can count on Agilent to support you at every step. Agilent’s infrastructure enables a delivery network that gets you what you need fast, anywhere in the world. That infrastructure also provides worldwide columns and chemistries technical support, as well as speedy problem resolution if you need it.