

WHAT CHARACTERISTICS WOULD A **PERFECT** SUPPORT FOR **ALL** SOLID PHASE SYNTHESIS NEEDS POSSESS?

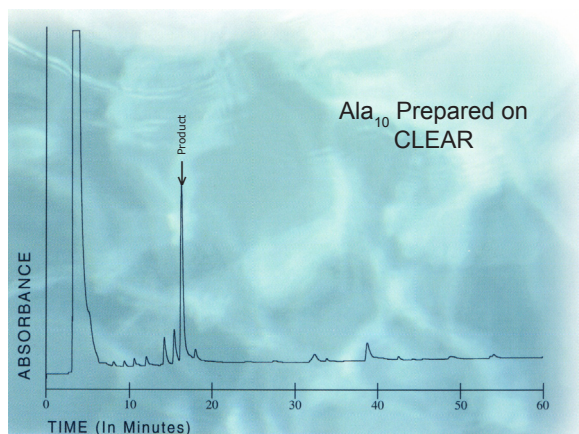
1. Inert matrix
2. Easily filtered
3. Deters aggregation
4. Good swelling properties
5. Promotes rapid reaction rates
6. Suitable for batch or continuous flow synthesis
7. Compatible with water for protein affinity matrix applications

The Answer is

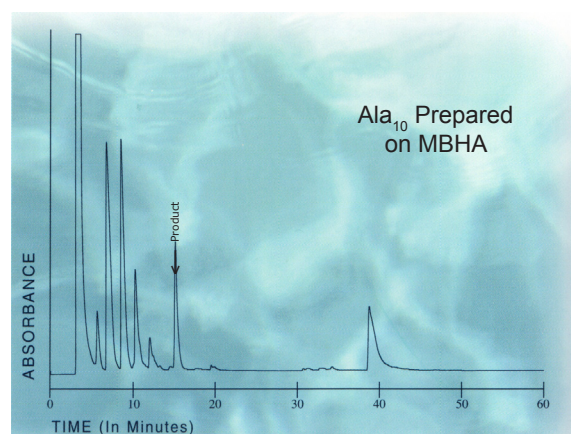
CLEAR™

CLEAR (Cross-Linked Ethoxylate Acrylate Resins) was developed by George Barany and Maria Kempe at the University of Minnesota.^{1,2,3} These products retain the highly desirable solvation properties of polyethylene glycol (PEG) or of PEG-linked products but with greater convenience. Unlike conventional liquid phase synthesis, developed by Bayer and Mutter in the 1970's⁴ and recently popularized by Janda in combinatorial synthesis,⁵ CLEAR resin is a highly cross-linked solid support. It is produced in a bead form using a large-scale polymerization process developed at Peptides International.⁶ The

CLEAR particles swell in a wide range of solvents including water, dichloromethane (DCM), or dimethylformamide (DMF). They are also compatible with relatively non-polar solvents such as tetrahydrofuran (THF) or dioxane. Synthesis can be performed on CLEAR in automated or manual synthesizers. Applications for multiple parallel synthesis and combinatorial chemistries are under development. Clearly, your own imagination should lead to many other uses for a product as exciting as CLEAR. Organic Synthesis?^{7,8} Affinity Chromatography? Enzyme Immobilization? Trace Analysis? Remote Sensor Applications? *Isn't it CLEAR what your next solid-phase support choice should be?*



Analytical HPLC chromatogram of crude H-(Ala)₁₀-Val-NH₂ prepared by batch-wise automated synthesis (Rainin, PS-3) on CLEAR-Amide resin. Synthesis performed using HBTU as coupling agent, four fold excess of Fmoc-Ala for 30 + 90 minutes (double coupling). HPLC performed on Vydac C₁₈ column (4.6 x 250 mm), gradient 5% →65% ACN/0.05% TFA in 60 minutes, 1 ml/min, 220 nm.



Analytical HPLC chromatogram of crude H-(Ala)₁₀-Val-NH₂ prepared by batch-wise automated synthesis (Rainin, PS-3) on 1% cross-linked polystyrene. Synthesis performed using HBTU as coupling agent, four fold excess of Fmoc-Ala for 30 + 90 minutes (double coupling). HPLC performed on Vydac C₁₈ column (4.6 x 250 mm), gradient 5% →65% ACN/0.05% TFA in 60 minutes, 1 ml/min, 220 nm.

TECHNICAL TIPS FOR CLEAR RESINS

General Information for Handling CLEAR Resins

CLEAR resins^{1,2,3} have been widely used in solid-phase synthesis, ranging in applications from on-resin disulfide formation to solid phase organic syntheses.^{7,8} Unlike other commonly used solid supports (PEG-PS or Tentagel), CLEAR resin particles are highly cross-linked, structurally uniform, and are generated from a large-scale suspension polymerization process (not grafted onto polystyrene). Attaining the best solvation conditions for solid phase synthesis is highly desirable. CLEAR resins by their very nature mimic the solubility

properties of polyethylene glycol (PEG) or PEG-linked products but with greater convenience. CLEAR particles swell in a variety of solvents, including water, DCM or DMF. These resin particles are also compatible with relatively non-polar solvents, such as THF or dioxane.

Recommendation: After completion of synthesis use hexane or diethyl ether as a final washing step before drying the resin. If MeOH or EtOH is used as a substitute, CLEAR resins (as well as other ethylene glycol-containing resins) can become gelatinous and may be more difficult to handle.

Cleavage Procedure for Fmoc-CLEAR-Acid Resins

Fmoc-CLEAR-Acid Resins are suitable for both batch and continuous flow synthesis utilizing standard Fmoc/t-butyl-based strategies. Cleavage and side-chain deprotection of the peptide-resin can be accomplished using TFA cocktails with appropriate scavengers depending on the peptide sequence and selection of side-chain protection. The most widely used combination of scavengers is reagent K⁹ [TFA: thioanisole: ethanedithiol: phenol: water (82.5:5:2.5:5:5)]. This mixture can be applied to most peptides. In the case of preparation of very hydrophobic peptides or other organic compounds, use of scavengers should be limited to those that would not be troublesome to remove during post-cleavage work-up.

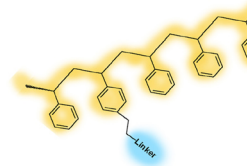
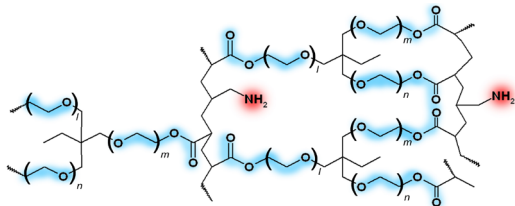
All widely used TFA-scavenger combinations can be applied to CLEAR resin.¹ A typical protocol for cleavage of 1 g of dry peptide-resin is carried out in 20-30 mL of TFA-scavenger mixture for 2 hours at room temperature. The resin is filtered and rinsed with TFA and the TFA is evaporated from the combined filtrates. The peptide is precipitated upon trituration with cold ether and is then collected by filtration or centrifugation and washed with cold ether to remove scavengers (Note: Please save the ether layer if the desired product is non-polar). The peptide should be dried in a desiccator in vacuo and then purified. In general it is recommended that small scale cleavage reactions may be carried out to optimize the best conditions for each preparation. Several review articles describing cleavage methods give more detailed procedures and should be consulted prior to synthesis.^{10,11}

1. M. Kempe and G. Barany, "CLEAR: A Novel Family of Highly Cross-Linked Polymeric Supports for Solid Phase Synthesis", *J. Am. Chem. Soc.*, **118**, 7083-7093, (1996).
2. M. Kempe and G. Barany, "Novel Highly Cross-Linked Polymeric Supports for Solid Phase Applications", in *Solid Phase Synthesis (Fourth International Symposium, Edinburgh Scotland)* R. Epton, Ed., Mayflower (London) 1996, pp. 191-194.
3. CLEAR products are protected under US Patents 5,910,554 and 5,656,707 granted to the Regents of the University of Minnesota.
4. E. Bayer and M. Mutter, *Nature (London)*, **237**, 512 (1972).
5. K.D. Janda, "Tagged versus Untagged Libraries: Methods for Degeneration and Screening of Combinatorial Libraries", *Proc. Natl. Acad. Sci. USA*, **91**, 10779-10785 (1994).
6. K. Darlak, I. Romanovska, A.F. Spatola, G. Barany, and M. Kempe, "A New Solid Support for Peptide and Organic Synthesis", Fifteenth American Peptide Symposium; Nashville, Tennessee, USA; June 1997.
7. N. Chatla, K. Darlak, and A.F. Spatola, "Applications of CLEAR Resin for Solid Phase Organic Synthesis: Comparison with Polystyrene Based Resin" *Innovation and Perspectives in Solid Phase Synthesis and Combinatorial Libraries (Fifth International Symposium, London, England)* R. Epton, Ed., Mayflower (London) 1998, pp. 275-276.
8. K. Darlak, M. Darlak, and A.F. Spatola, "Disulfide Oxidation in Water: Investigation of CLEAR Supports for On-Resin Cyclization" *Peptide Science - Present and Future (Proceedings of the 1st International Peptide Symposium, Kyoto, Japan)* Y. Shimonishi, Ed., Kluwer Academic Publishers (Dordrecht) 1997, pp. 584-586.
9. D.S. King, C.G. Fields, and G.B. Fields, *Int. J. Peptide Prot. Res.*, **36**, 255 (1990).
10. G. Fields and R. Noble, *Int. J. Peptide Prot. Res.*, **35**, 161 (1990).
11. F. Dick, In *Methods in Molecular Biology*, Volume 35, Peptide Synthesis Protocols, Eds., Pennington, M. W. and Dunn, B. M., Humana Press, New Jersey; 1994; pp. 63-72.

FREQUENTLY ASKED QUESTIONS

Q. How do CLEAR resins differ structurally from traditional polystyrene supports?

- A.** First, there are no aromatic rings in CLEAR base resins. As a result, there are no large blocks of hydrophobic structure that can result in unwanted aggregation with lipophilic materials. Most of the core CLEAR structure is composed of ethylene glycol (-CH₂-CH₂-O-)n units, making it more ether-like in its overall solubility characteristics. The rest of the structure (see diagram below for comparison between polystyrene and CLEAR) is made up of a cross-linking unit (which makes it a solid) and an amine-containing moiety (which provides the handle for further structural elaboration and solid phase synthesis).



$$l + m + n \sim 14$$

Q. Is CLEAR compatible with combinatorial chemistry and for solid phase organic synthesis?

- A.** Yes, to both parts. CLEAR resins have several advantages for a wide range of organic reactions in view of the fast reaction rates, ether-like structure and absence of an aromatic core component.

Q. I have noticed the TFA cleavage mixture is somewhat difficult to filter because the resin is in such an extremely swollen state. Any suggestions?

- A.** While other non-polystyrene-based resins are sold in slurries, CLEAR resins are sold in dry, solid form and possess excellent swelling properties. For faster filtration times and easier manipulation of CLEAR, we recommend using a wide-diameter, glass buchner funnels after final TFA cleavage.

Q. What is the typical procedure to load the first amino acid onto CLEAR resin?

- A.** The same standard published methods that are suitable for Wang resins can also be used to load the first amino acid onto CLEAR resins.

Q. Do coupling reactions occur faster on CLEAR?

- A.** Because of its macroporous nature, CLEAR resins are less subject to diffusion limited processes. While CLEAR is not a membrane support, it does offer many of the advantages of membrane-based peptide synthesis. This can reduce the times for some coupling reactions.

Q. How does CLEAR compare with TentaGel and PEG-PS?

- A.** For one, it's usually less expensive! But the major difference is that TentaGel™ and PEG-PS™ are composed of poly(oxyethylene) units but these are attached to a polystyrene core. CLEAR has no polystyrene in its structure. And CLEAR is compatible with water-containing solvent mixtures.

Q. Can I use CLEAR for protein purification based on affinity chromatography?

- A.** Because of its compatibility and good swelling characteristics, CLEAR resins should be ideal for use with proteins in water mixtures. CLEAR resin does not exactly swell in pure water. However, if it is first pretreated with an organic solvent such as DMF and then water content is gradually increased, it will perform very well with aqueous solvent mixtures and buffers. We believe this will be one of the most useful applications of CLEAR resins.

Q. What other functional groups are present in the CLEAR core?

- A.** Besides the amine or functionalized groups (if you select some of the modified CLEAR resins), the only other organic moieties present within the CLEAR structure are the ether of the ethylene glycol and an alkyl ester from the trifunctional monomer.

Q. Can side reactions with acid or base lead to decomposition of the CLEAR support?

- A.** Generally, no. Because of the ladder-like structure of any highly cross-linked matrix, one would have to break numerous bonds before significant deterioration could occur.



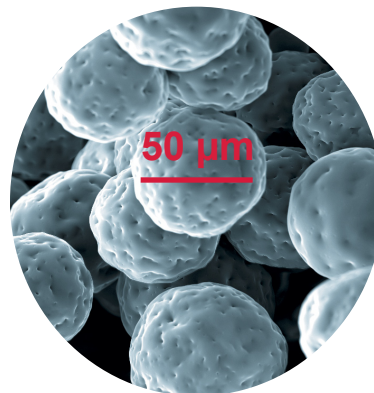
Q. What is the molecular weight cut-off for CLEAR resin? Does it somewhat vary from lot to lot?

A. CLEAR is prepared by a suspension polymerization technique which is carefully performed to provide a uniform product. While specific molecular weight cutoffs are not yet available for the base or functionalized CLEAR resins, we have prepared relatively large peptides with no apparent accessibility problems. Further studies to answer this question are in progress.

Q. What is the typical size of the CLEAR particles?

A. CLEAR resins are carefully passed through sieves in order to insure particle uniformity. The result is that the final product is a spherical bead with a diameter of 90-250 μm .

SEM (Scanning Electron Micrograph) of CLEAR resin particles: The structure is nearly 100% cross-linked. The polyethylene glycol backbone makes the resin fully accessible to a wide range of solvents and reagents including aqueous solutions. Unlike liquid phase PEG resins, CLEAR particles are easy to filter and resistant to all but the most harsh acid or base solutions.



Q. What is the usual substitution value for CLEAR-Base Resin (HCl), for CLEAR-Acid Resin and for CLEAR-Amide Resin?

A. The substitution level of CLEAR-Base Resin (HCl) is usually between 0.2 and 0.7 meq/g. The acid and amide resins typically range from 0.2 to 0.6 meq/g based on elemental or spectrophotometric analysis.

Q. Are CLEAR resins compatible with most automated machines? How can they be agitated?

A. CLEAR resins have been used in vessels with wrist action (rocking) shakers, with nitrogen agitation and with slow overhead paddle stirring (less than 30 rpm). Magnetic stirring is generally not recommended for CLEAR resins or for any other fine resin product. CLEAR resins are also ideal for continuous flow applications.

Q. The resin is more difficult to handle than other resins commonly used. Any suggestions on how to minimize difficulties?

A. Most of the core CLEAR structure is composed of ethylene glycol (-CH₂-CH₂-O-)n units. This property renders the resin less free-flowing than polystyrene-based resins and can increase "stickiness." One suggestion is to avoid using wax paper and try to minimize transfer steps. Instead weigh CLEAR resins directly into your solid phase synthesis glassware or other vessel. It is also highly recommended that glassware used in synthesis be silanized prior to use (SurfaSil™ available from Pierce).

Q. Prior to synthesis should the resin be pre-treated?

A. Again, because of its macroporous nature, CLEAR resins are less subject to diffusion limited processes. However, in solid phase synthesis resins, are typically pre-swelled in an organic solvent to improve accessibility within their macroporous structure.

Q. What is the procedure for neutralizing the CLEAR-Base Resin (HCl)?

A. Pre-swallow the resin in DCM for approximately 30-60 min. Perform 2 neutralization washes with 10% TEA/DCM for 2 min and 5 min. Then perform the following sequence of washes 2 times: 3 min washes in DCM and followed by DMF. The resin is now ready for coupling.

Swelling Properties of CLEAR-Base Resin	
Solvent	Bed Volume (ml) of 1 g of resin
CH ₂ Cl ₂	7.0
DMF	6.5
THF	7.0
MeOH	6.0
H ₂ O	5.5



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