PreACT™ Activated Resins

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United States & Canada **800-207-0144**
Germany **0800 6931 000**
United Kingdom **0800 622935**
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PreACT Activated Resins

PreACT Agarose ALD Kit	10 ml	70695-3
	50 ml	70695-4
PreACT Fractogel® AZL	15 g	70697-3
PreACT Fractogel EPX	15 g	70696-3

PreACT Agarose ALD

PreACT™ Agarose ALD is an activated chromatography matrix for simple and efficient immobilization of affinity ligands under mild conditions. Small ligands to large proteins can be immobilized to form stable affinity resins. The coupling reaction is performed under physiological conditions and all necessary components are provided. PreACT Agarose ALD is supplied in an activated form containing bound reactive aldehyde groups. During ligand coupling, aldehydes react with primary amines on the ligand to form a Schiff-base. In a further reaction, the Schiff-base linkage is selectively converted into a stable, secondary amine linkage. The highly stable linkage extends the functional life of the prepared affinity resin and dramatically reduces ligand leaching in comparison to other immobilization methods such as cyanogen bromide. PreACT Agarose produces affinity resins that can be reused many times without significant loss of capacity.

PreACT Fractogel AZL

PreACT Fractogel AZL is an activated resin suitable for the coupling of proteins under physiological conditions. The Fractogel resins are based on a 20–40 µm polymethacrylate polymeric bead to which linear polyelectrolyte chains ("tentacles") have been grafted. Fractogel resins are stable to a broad pH range (2–12), denaturants such as guanidine HCl and urea, detergents and organic solvents. PreACT Fractogel AZL has a high density of tentacle-bonded azlactone reactive groups. The azlactone ring is opened by nucleophilic attack of appropriate groups present on the protein surface. Upon reaction with amines, a stable amide bond forms between the former carbonyl function of the azlactone. Coupling is performed simply by incubation with the ligand in an appropriate buffer. The resin is supplied as a dry powder.

PreACT Fractogel EPX

PreACT Fractogel EPX is an activated resin for the immobilization of low molecular weight amine bearing ligands or alkaline-stable proteins. Based on the same Fractogel polymeric bead with grafted polymers described above, the functional activated epoxy groups are bonded to the tentacles resulting in improved ligand density and reduced steric interference. Bonded epoxide groups react with primary amino, hydroxyl and sulfhydryl groups to form stable ether linkages. Coupling is performed simply by incubation with the ligand in an appropriate buffer. The resin is supplied as a dry powder.

Components

PreACT Agarose ALD Kit

- 10 ml or 50 ml PreACT Agarose ALD (50% v/v slurry)
- 1 or 2 10X PBS Buffer Tablet(s)
- 2.5 ml or 12.5 ml 1 M Sodium Cyanoborohydride
- pkg/5 or pkg/10 Chromatography columns

Storage

Store PreACT Agarose ALD Resin at 4°C. Resin is supplied as 50% v/v slurry in 20% ethanol.

Store dry PreACT Fractogel AZL or EPX Resins at –20°C.

Store 10X PBS Buffer Tablets at room temperature

Store Sodium Cyanoborohydride solution at 4°C.

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Using PreACT Resins

Table 1. Summary of PreACT resin properties

Property	PreACT Agarose ALD	PreACT Fractogel® EPX	PreACT Fractogel AZL
Activated group	Aldehyde	Epoxy	Azlactone
Ligand reactive groups	RNH_2	RNH_2, RSH, ROH	RNH_2, RSH, ROH
Bonds formed	dialkylamine (R–NH–R) (+Na cyanborohydride)	alkylamine, thioether, ether	amide (primarily)
Coupling buffer (typical)	phosphate (PBS)	phosphate, carbonate (20–50 mM)	phosphate (20–50 mM) 150 mM NaCl
Ligand concentration in coupling reaction	1–5 mg/ml	up to 40 mg/ml[a] 5 mg/ml[b]	5 mg/ml
pH of reaction	7–8	8–12, optimal > 9[a] 8.5–9[b]	7–8
Reaction temperature	4–25°C	25–40°C[a] 4–25°C[b]	4–25°C
Reaction time	2–24 h	2–5 days[a] 1–5 days[b]	0.5–3 h
Reaction volume	4 ml/ml settled resin	20 ml/g resin	20 ml/g resin
Ligand density (max.)	> 4 mg/ml settled resin	>1.5 µmol/g[a] (1 g = 3.5 ml resin)	0.1 µmol/g (1 g = 3.5 ml resin)

a. For low molecular weight ligands (stable to alkaline hydrolysis)
b. For proteins

Ligand requirements

PreACT resins are suitable for the immobilization of any molecule that has an available, non-aryl primary amine functional group. PreACT EPX and AZL ar also capable of reacting with sulfhydryl and hydroxyl groups. For protein targets, the majority of amines are contributed by the epsilon amine of lysyl side chains. The amino terminus is also available for coupling. The protein must be in a buffer lacking primary amines. The presence of reducing agents, azide, Tris or other primary amine components can inhibit the coupling reaction. Likewise, ligands that are dissolved in solvent prior to immobilization should be dissolved in amine free buffers. If there is doubt about the counterion or components in a dry or suspended ligand, it is advisable to dialyze the ligand against the recommended coupling buffer in each PreACT protocol. The protocols below require a protein stock solution having a concentration of 5 mg/ml or higher. If the target protein is below this concentration, the total reaction volume can be increased as required although reaction rates may be lower.

Recommended protein amounts

The highest efficiency coupling reactions require an optimized ratio of protein:resin. This ratio of protein:resin will generally result in 80–100% coupling and consume the available resin reactive groups, eliminating the need for end-capping of the resin. While lower protein:resin ratios can be used, end-capping may be required to eliminate residual reactive groups on the resin. Using higher protein:resin amounts will result in more rapid coupling and complete consumption of resin reactive groups. However, it will also result in a higher amount of residual, uncoupled protein that must be removed prior to chromatographic use of the resin. For small or non-protein ligands, some empirical testing may be required to establish optimal ligand:resin ratios.

Resin	Protein binding capacity (per ml resin)
Fractogel EPX	10–20 mg per ml settled resin
Fractogel AZL	10–20 mg per ml settled resin
Agarose ALD	4–6 mg per ml settled resin

Monitoring reaction progress

The easiest method for monitoring reaction progress is to measure unbound protein over the course of the reaction. The level of protein in the aqueous phase can be determined by optical absorbance measurements at 280 nm or by enzymatic or other assay, where applicable. Immediately following the initiation of a coupling reaction, remove twice the cuvette volume of the reaction containing the suspended resin and transfer to a microcentrifuge tube. Spin at 10,000 rpm for 30 sec. Carefully remove the supernatant, being careful not to disturb the resin bed. Read the A_{280} of this sample using coupling buffer as a blank and a thoroughly washed cuvette. When performing small-scale coupling reactions, the resin and supernatant can be returned to the coupling reaction to avoid significant losses. Over the course of the reaction, the A_{280} of the supernatant will decrease in proportion to the amount of protein coupled. The reaction is complete when no changes in absorbance are observed. The change in absorbance can be used to calculate the precise amount of protein immobilized. For example, if a coupling reaction was initiated at 3 mg protein/ml resin, the initial A_{280} was 1.0 and the final A_{280} was 0.181, the percentage of coupling is:

$$\frac{1.0 - 0.181}{1.0} \times 100 = 82\%$$

thus, 82% × 3 mg protein/ml resin = 2.46 mg protein/ml resin coupled

Commonly used protein assays such as Bradford and BCA are not recommended for monitoring the PreACT Agarose ALD reaction due to the interference caused by reducing reagent (sodium cyanoborohydride) present in the coupling reaction. These assays are suitable for monitoring protein concentration when using either the Fractogel AZL or EPX resins.

Small ligand coupling reactions can be monitored by spectrophotometer provided the target ligand has reasonable molar absorbtivity in the UV or visible range. Other possible monitoring methods might include HPLC, mass spectrometry, ELISA, etc. The only requirement is that the assay method be capable of detecting a change in the analyte concentration using only very small amounts of sample or be nondestructive so reaction supernatants can be returned to the reaction.

Reaction Protocols

The reactions can be scaled to the level of available protein/ligand and are described on a 5 ml settled resin scale (10 ml slurry) as an example only. The batch coupling protocol allows for faster reaction kinetics, easier reaction monitoring, and a more homogeneous immobilization reaction. This is the preferred protocol for most applications. For convenience, the batch coupling can be performed "in column". Samples of the reaction supernatant can be collected simply by opening the stopcock. This allows for faster more convenient reaction monitoring without centrifugation.

PreACT Agarose ALD

1. Dissolve the supplied 10X PBS Buffer Tablet in sterile deionized water to a final volume of 100 ml to make a 10X PBS stock solution. Stir until tablet is completely dissolved then use promptly, autoclave, or sterilize by filtration. Phosphate buffers are prone to bacterial growth and autoclaving is recommended for long term storage. Do not use sodium azide or other bacteriostatic reagent for this stock. The presence of azide or other components can interfere with reaction chemistry.
2. Prepare ligand for immobilization following the guidelines in the “Ligand requirements” section, above.
3. Determine the reaction scale desired, using the “Recommended protein amounts” section, above. Completely suspend the 50% PreACT ALD Agarose slurry and remove 2X the required resin volume (e.g. for a 5 ml bed volume, remove 10 ml of 50% slurry). Transfer to a clean tube and wash twice with 5 bed volumes of deionized water: allow resin to settle or spin at 1000 × g for five min. Remove the supernatant and discard. Resuspend the resin in the original volume of deionized water to make a 50% slurry.

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4. Assemble the following components in the order listed for a 20 ml coupling reaction:
   10 ml PreACT ALD Agarose 50% slurry (resuspended resin from step 3)
   (6–x) ml deionized water
   2 ml 10X PBS prepared in step 1 (1/10th of total reaction volume)
   x ml (20–30 mg) ligand/protein
   2 ml 1 M Sodium Cyanoborohydride (1/10th of total reaction volume)
   20 ml total volume
5. Invert the tube several times to thoroughly mix components. Immediately remove an aliquot and determine initial ligand A_{280} according to the guidelines in "Monitoring reaction progress", above. If desired, return sample aliquot to the reaction. Place tube on shaking platform or rotating wheel and incubate at 4–25°C.
6. Monitor the reaction periodically to assess coupling. Most proteins are coupled within 24 h at 4°C, or within 2 h at 25°C.
7. Upon completion of the reaction, if end-capping is desired, add end-capping reagent to the reaction and incubate as described in the "Optional end-capping protocol", below.
8. Wash the resin at least twice with 5 bed volumes of 1X PBS: allow resin to settle by gravity, or spin at 1000 × g for five min. Remove supernatant.
9. Resin must be cycled with the applicable binding and elution buffers prior to use. For example, if an antibody ligand was immobilized and affinity purification called for incubation in PBS followed by 0.2 M glycine elution buffer, the resin must be cycled through these buffer conditions to remove any uncoupled protein that might otherwise be liberated during use.
10. Equilibrate the resin in 1X PBS or other suitable buffer supplemented with a bacteriostatic agent. Store at 4°C, protect from freezing.

PreACT Fractogel® EPX

The buffer used for coupling must be free of Tris-HCl, glycine or any nucleophilic components. Phosphate, borate or carbonate buffers work well. The buffer pH can be adjusted with NaOH. The choice of pH will depend on a number of factors including the stability of the ligand and the length of incubation. Epoxy groups react more rapidly at higher pH, but the competing hydrolysis rates are also increased.

1. Prepare 60 ml coupling buffer (100 mM sodium carbonate or sodium phosphate pH 9–11).
2. Warm sealed bottle of PreACT Fractogel EPX Resin to room temperature before opening. Weigh out 3 g of resin. Cap remaining resin and store at 4°C. Storage in a dessicator will extend the life of the resin. 3 g of resin will expand to approximately 10 ml settled volume.
3. Suspend resin in 30 ml coupling buffer and allow resin to swell for 10 min.
4. Add 100 mg protein or ligand in coupling buffer.
5. Add coupling buffer to a final volume of 60 ml. Cap and invert the tube several times to thoroughly mix components. Immediately remove an aliquot and determine initial ligand A_{280} (if protein) according to the guidelines in "Monitoring reaction progress", above. If desired, return sample aliquot to the reaction and place on shaking platform or rotating wheel. Incubate at 4–25°C for 24 hr.
6. Monitor the coupling reaction to assess coupling efficiency. Continue incubation until the protein concentration in the supernatant stops decreasing. This may require total incubation times of up to 120 h, especially at lower tempertures.
7. Upon completion of the reaction, if end-capping is desired, add end-capping reagent and incubate as described in the "Optional end-capping protocol" below.
8. Wash the resin at least twice with 5 bed volumes of 1X PBS (137 mM NaCl, 3 mM KCl, 4 mM Na_2HPO_4, 1 mM KH_2PO_4 pH 7.4).
9. Resin must be cycled with the applicable binding and elution buffers prior to use. For example, if an antibody ligand was immobilized and affinity purification called for incubation in PBS followed by 0.2 M glycine elution buffer, the resin must be cycled through these buffer conditions to remove any uncoupled protein that might otherwise be liberated during use.

10. Equilibrate the resin in 1X PBS or other suitable storage buffer supplemented with a bacteriostatic agent. Store at 4°C, protect from freezing.

PreACT Fractogel® AZL

1. Prepare 60 ml 1X PBS (137 mM NaCl, 3 mM KCl, 4 mM Na_2HPO_4, 1 mM KH_2PO_4 pH 7.4).
2. Warm sealed bottle of PreACT Fractogel AZL Resin to room temperature before opening. Weigh out 3 g of resin. Cap remaining resin and store at 4°C. Storage in a dessicator will extend the life of the resin. 3 g of resin will expand to approximately 10 ml settled volume.
3. Suspend resin in 30 ml 1X PBS and allow resin to swell for 10 min.
4. Add 100 mg protein or ligand in a coupling buffer.
5. Add 1X PBS to a final volume of 60 ml. Cap and invert the tube several times to thoroughly mix components. Remove an aliquot and determine initial ligand A_{280} (if protein) according to the guidelines in "Monitoring reaction progress", above. If desired, return sample aliquot to the reaction and place on shaking platform or rotating wheel. Incubate at 4–25°C for 30 min.
6. Monitor the coupling reaction to assess coupling efficiency. Continue incubation until the protein concentration in the supernatant stops decreasing. This may require total incubation times of up to 3 h.
7. Upon completion of the reaction, if end-capping is desired, add end-capping reagent and incubate as described in the "Optional end-capping protocol" below.
8. Wash the resin at least twice with 5 bed volumes of 1X PBS.
9. Resin must be cycled with the applicable binding and elution buffers prior to use. For example, if an antibody ligand was immobilized and affinity purification called for incubation in PBS followed by 0.2 M glycine elution buffer, the resin must be cycled through these buffer conditions to remove any uncoupled protein that might otherwise be liberated during use.
10. Equilibrate the resin in 1X PBS or other suitable storage buffer supplemented with a bacteriostatic agent. Store at 4°C, protect from freezing.

Optional end-capping protocol

Under normal conditions, end-capping is unnecessary. However, when performing protein coupling to PreACT resins at very low protein:resin ratios, end-capping is a means of chemically blocking the excess reactive groups (if any) on the resin. Before adding any end-capping reagent, be certain that the ligand coupling reaction has gone to completion. If there is measurable ligand in the supernatant after the incubation period specified in the protocol, end-capping is unnecessary. If 100% of the available ligand has been coupled, any remaining reactive groups can be capped by treatment with one of the following reagents:

PreACT Resin	End-capping reagent	Method
Fractogel EPX	0.2 M ethanolamine, pH 9.5	Incubate 1–3 h at 4–21°C with gentle agitation. Then proceed with the washing procedures described in the protocols above
Fractogel AZL	0.2 M glycine, pH 8.0	
Agarose ALD	0.2 M ethanolamine, pH 8.0 or 100 mM Tris-HCl, pH 8.0	

Note: The use of BSA or other protein-based end-capping reagents is not recommended. Bulky adducts can cause steric hindrance and reduce resin performance.

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Recommended storage conditions

PreACT resins that have been reacted with protein should be stored at 4°C in the presence of a bacteriostatic agent such as 20% ethanol, 0.02% thimerosal, or 0.02% sodium azide. Fractogel resins do not support microbial growth, but immobilized proteins can act as a growth medium. The recommended buffer will depend on the ligand immobilized. The main objectives when choosing a storage buffer are to stabilize the ligand molecule (compatible pH, ionic strength, cofactors, metals, etc.) and to prevent bacterial growth. For many applications such as antibody coupling, the resin can be stored in 1X PBS, pH 7.4, 0.02% sodium azide. These conditions will maintain antibody stability and allow resin use without prior equilibration. Hydrated PreACT resins should be protected from freezing since this can reduce flow rates.

Related Products

Product	Size	Cat. No.
Ion Exchange Resins		
Fractogel EMD TMAE (M)	50 ml	70698-3
Fractogel EMD TMAE (S)	50 ml	70699-3
Fractogel EMD TMAE Hicap (M)	50 ml	70700-3
Fractogel EMD DEAE (M)	50 ml	70701-3
Fractogel EMD DEAE (S)	50 ml	70702-3
Fractogel EMD DMAE (M)	50 ml	70703-3
Fractogel EMD DMAE (S)	50 ml	70704-3
Fractogel EMD SO_3^- (M)	50 ml	70705-3
Fractogel EMD SO_3^- (S)	50 ml	70706-3
Fractogel EMD SE Hicap (M)	50 ml	70707-3
Fractogel EMD COO^- (M)	50 ml	70708-3
Fractogel EMD COO^- (S)	50 ml	70709-3
Thiophilic Adsorption Resin		
Fractogel EMD TA (S)	25 ml	70712-3
Hydrophobic Interaction Resins		
Fractogel EMD Propyl (S)	50 ml	70710-3
Fractogel EMD Phenyl (S)	50 ml	70711-3
Size Exclusion Resin		
Fractogel EMD BioSEC (S)	150 ml	70715-3