Affi-Prep® 10 Affinity Chromatography Support

Instruction Manual

Catalog Numbers
156-0001
156-0002
156-0003

BIO-RAD

Table of Contents

Section 1
Description

The Affi-Prep 10 support is ready to use for immobilization of proteins, peptides, and aminated nucleic acids. It is activated with N-hydroxysuccinimide esters and used to immobilize ligands, such as proteins and peptides, through primary amino groups, forming stable amide bonds.

The Affi-Prep 10 support is exceptionally easy to use; the ligand to be immobilized is mixed with the support in appropriate buffer or organic solvent for 1–4 hours. The resulting affinity support is stable, with high flow rates, low ligand leakage and low non-specific interaction, even under high salt elution conditions. The typical binding capacity is 10–15 mg of human IgG per ml of support.

Section 2
Technical Description

The Affi-Prep 10 support is based on macroporous methacrylate co-polymer beads. It is activated with an N-hydroxysuccinimide ester on a 10-carbon arm. The NHS ester reacts rapidly with primary amines on proteins and peptides to form stable amide bonds.

It is used in buffered aqueous solutions or organic solvents, and proteins typically couple with high efficiency within 1–3 hours at 4 °C. The support is supplied in 100% isopropanol, which is removed prior to coupling. Table 1 lists properties of the Affi-Prep 10 support.

Table 1. Characteristics Of The Affi-Prep 10 Affinity Chromatography Support

Base material	Methacrylate
Activation chemistry	N-hydroxysuccinimide
Specificity	Primary amines
Typical binding capacity	10–15 mg human IgG/ml support
Coupling buffers	MES, MOPS, HEPES, POPSO, acetate, and bicarbonate
Nominal particle size	50 µm
Nominal pore diameter	1,000 Å
Autoclavable	121 °C, 30 min. (ligand permitting)
Stability after coupling	pH 2–10, alcohols, urea, detergents, commonly used buffers
	Do not use isothiocyanates
Storage	
Before coupling	-20 °C or colder
After coupling	4° C, and/or 0.02% sodium azide, or 20% ethanol
Shelf life	12 months at -20 ˚C

Section 3
Coupling Conditions

The Affi-Prep 10 support couples proteins best at a pH below their isoelectric points. Hydrolysis of some active ester groups occur during aqueous conditions to generate carboxylate ions. This will give a slightly negative charge to the gel which will enhance the coupling of positively charged proteins.

The support is also available prepacked in an HPLC cartridge. The cartridge is washed free of isopropanol with buffer, and the buffered ligand syringed over the cartridge several times. The cartridge is recommended for highest coupling efficiency, especially when quantities of ligand are limited.

Coupling can be performed under very mild conditions, at pH 3–10. To maintain pH control a minimum buffer strength of 50 mM is recommended. Suitable coupling buffers include MES, MOPS, HEPES, POPSO, acetate, and bicarbonate. Buffers which contain amine groups, such as Tris, Bis-Tris, or glycine, or buffers containing azide should be avoided during coupling, but may be used with the coupled gel.

Phosphate buffers have been used with some success but may interfere with the coupling.

Section 4
Coupling

Coupling can be done in a batchwise mode, or in a column. Large volumes of support are more easily coupled in a column than batchwise. The gel is washed free of isopropanol using cold buffer, the buffered ligand added, and the reaction allowed to proceed. After the coupling is completed excess ester groups are blocked. Uncoupled protein and released N-hydroxysuccinimide ester are then eluted with binding buffer. Coupling at 4 °C is recommended whenever possible, particularly when coupling sensitive biopolymers.

Table 2. Summary Of Coupling Conditions

Concentration of ligand	
Protein	Optimum 5–10 mg/ml of support Min. 0.5–1 mg/ml, Max. 20 mg/ml
Low MW ligand	10–15 µmoles/ml
pH	Near or below pI of ligand; for acidic proteins, add 80 mM $CaCl_2$
Aqueous buffers	MES, MOPS, HEPES, POPSO, acetate, bicarbonate (avoid Tris and glycine)
Organic solvents	Alcohols, DMSO, dioxane, acetone, formamide
Temperature	4–8 ˚C recommended
Coupling time	1–4 hours
pH range	2–12
Reaction volume (batch)	1.5–4.5 ml of ligand solution per ml of support
Compatible buffers (derivatized support)	10 mM DTT, non ionic detergents, urea, Guanidine-HCl, ethylene glycol

4.1 Batch Coupling Procedure

1. Slurry the support to a uniform suspension.
2. Transfer the desired amount to a Buchner funnel, or similar device.
3. Wash under vacuum with 30–50 volumes of cold 10 mM sodium acetate, pH 4.5. Take care to maintain a moist cake. For optimum coupling efficiency, complete the washing within 15 minutes.

4. Transfer the support to a reaction vessel, and add cold ligand solution (see Section 3). Add at least 0.5 mg of ligand per ml of support. It is recommended that the ligand be dialyzed against the coupling buffer to remove smaller amine containing contaminants.
5. Agitate gently on a rocker, shaker, or wheel for at least 1 hour at 20 ˚C (room temperature) or 2–4 hours at 4–8 ˚C. The reaction can be allowed to proceed overnight if desired.
6. Block any remaining active esters with either 0.1 ml of 1 M glycine ethyl ester, pH 8.0, or 0.1 ml of 1 M ethanolamine, pH 8.0, per ml of support. The blocking step is very rapid, and will only take a few minutes.
7. Wash the gel with 2–4 volumes of 0.5 M NaCl to remove uncoupled ligand.
8. Transfer the coupled support to a column and rinse with PBS, pH 7.2, until the $O.D._{280}$ of the effluent is stable.
9. Equilibrate the column with sample buffer. The column is now ready to use.

4.2 Column Coupling

1. Prepare 20 bed volumes of cold 10 mM sodium acetate, pH 4.5, rinsing buffer, and at least 8–10 bed volumes of cold coupling buffer. Dissolve your ligand in coupling buffer (1/2 bed volume or less), recommended concentration 5–20 mg of ligand/ml of buffer, or proportions which you have developed.
2. Perform the coupling in a cold room. Pour some rinsing buffer into the column and purge the bed support of air. Decant the settled support and slurry it in cold rinsing buffer. Pour the Affi-Prep 10 support into the column. Let it settle for a little bit with the outlet closed to start forming a bed. Drain off the isopropanol, without letting the bed run dry. Close the outlet, pour rinsing buffer into the column, and attach the flow adaptor. Connect to pump and open the outlet. Pump at least 10 bed volumes through the column, as fast as your system permits to minimize rinsing times. For optimum coupling efficiency, complete the washing in less than 15 minutes. Adjust the flow adaptor as the bed packs.
3. Change to coupling buffer, and equilibrate the column until the pH is stable, (4–5 volumes). For

optimum coupling efficiency this step should be completed within 20 minutes at 4–8 ˚C.

4. Start pumping the ligand solution through the column. Loop the inlet and the outlet, so that your ligand solution is recycled over the column for 4 hours, with minimal dilution.
5. Estimate the amount of protein coupled to the bead by mass balance calculations, either by monitoring the $O.D._{280}$ of the recycled ligand, or by protein assay. Coupling can be extended to overnight if desired.
6. Block remaining active esters through pumping 4–5 bed volumes of 0.1 M glycine ethyl ester, pH 8.0, or 0.1 M ethanolamine, pH 8.0, for 1 hour.
7. Rinse the column with 2–3 bed volumes of 0.5 M NaCl, to remove excess ligand and blocking agent.
8. Rinse the bed with PBS, pH 7.2, until $O.D._{280}$ is stable, or equilibrate the column with running/ sample buffer until pH and $O.D._{280}$ base line are stable.

Section 5
Coupling Efficiency

The efficiency of the coupling will vary with the protein being coupled. Ligand concentrations over 20 mg/ml of support can be used, but are not recommended, since the coupling efficiency decreases. Maximum coupling efficiency is obtained with ligand concentrations in the range 5–15 mg/ml of support, and with total reaction volumes between 1.5–4.5 ml ligand solution/ml of support. Unbound protein remaining in the coupling and washing buffers can be assayed using the Bio-Rad Protein Assay (catalog number 500-0002), or by $O.D._{280}$. If $O.D._{280}$ is used, the pH of the sample must be adjusted to < 2. Above pH 2, N-hydroxysuccinimide released during coupling will adsorb. N-hydroxy-succinimide also interferes with the Lowry Protein Assay.

Section 6
Elution

The support can be used with different elution strategies; specific elution, with excess of competitive inhibitor, antibody or antigen; acid elution, e.g. glycine-HCl, pH 2.5, 20 mM HCl, or sodium citrate, pH 2.5–3; base elution, e.g. 1 M NH_4OH or 50 mM diethyl amine, pH 11.5; or 8 M urea, or with chaotropic agents, such as 6 M guanidine hydrochloride.

Section 7
Regeneration/Cleaning

To remove tightly bound proteins following a separation, the column should be washed with 3 column volumes of 8 M urea, or 6 M guanidine hydrochloride, until the base line is stable.

Section 8
Sanitization

A combination of alcohol and detergent is a good way to sterilize and clean affinity media. The column can be equilibrated with 2% digluconate in 20% ethanol, allowed to incubate for 6–8 hours, and then rinsed extensively with sterile filtered application buffer.

Section 9
Storage

The uncoupled gel should be stored at -20 ˚C or lower for up to 12 months. For long term storage of the coupled affinity gel, depending on ligand stability we recommend the regeneration and sanitation described above, and storage of the gel refrigerated in either 20% ethanol, or 0.02% sodium azide.

Section 10
Warning

Due to the polymeric nature of the support, it should not be used with oxidizing agents such as 4 M nitric acid.

Section 11
Technical Assistance

For additional information and technical assistance, contact your local Bio-Rad representative, or, in the USA, call 1-800-4BIORAD.

Section 12
Product Information

Catalog Number	Product Description
156-0002	Affi-Prep 10 Support, 25 ml
156-0001	Affi-Prep 10 Support, 4 x 25 ml
156-0003	Affi-Prep 10 Support, 500 ml
125-0450	Affi-Prep 10 Cartridge, 30 x 4.6 mm
125-0452	Affi-Prep 10 Cartridge, 15 x 25 mm
To Measure Binding Efficiency	
500-0001	Bio-Rad Protein Assay, Kit 1
500-0002	Bio-Rad Protein Assay, Kit 2

Bio-Rad Laboratories, 2000 Alfred Nobel Dr., Hercules, CA 94547
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