FT-56408

Amine Reactive Agarose

Product Description

Amine reactive gel, for the preparation of peptides and proteins affinity support.

	Ami.R Gel	Ami.R. Gel Fast flow
Cat.number:	UP56408A, 5ml	UPR2289A, 5ml
Matrix:	Agarose 4% reticulated	Agarose 6% reticulated
Molecular Exclusion Limit:	20 Million Daltons	6 Million Daltons
Flow rate maximum :	250 cm/h	2000 cm/h
Bead size:	60-160µm	
Monoaldehyde group concentr.:	40-50µmoles/ml	
Spacer arm:	5 atoms, hydrophilic	

Benefits:
High binding capacity (up to 60 mg/ml BSA)
Higher activity of immobilized antibodies than with "oriented" coupling
Amine specificity (couple on NH2-bearing molecules!)

High coupling efficiency (85-99%), whatever is MW and pI !
Coupling compatible at pH3-10, with temperatures 0-40°C,
Fast coupling (in 20 minutes to 2 hours)
No endcapping necessary
Undetectable leaching of ligand (<0.1 ppm)

High flow rate (up to 250 cm/h)
Sanitizable with NaOH, Autoclavable
Drug Master File

Form: Suspension in deionized water containing 0.02% sodium azide or 20% ethanol

Storage: Store at +4 C. DO NOT FREEZE (H)

Applications: Preparation of immobilized ligand (antibody) gels for
-affinity purification,
-immunoprecipitation,
-affinity depletion…

Introduction

Uptima Amine Reactive Gels are optimal matrices for immobilizing small ligands to large proteins through their Amine groups. All are convenient to work with. Coupling is simple, rapid and highly efficient. Ami.R. Gel is favoured in research applications for its efficiency and ease of use. Diagnostic applications also benefit from its high binding efficiency, capacity, and ease of use (especially in manufacturing). In pharmaceutical processing, Ami.R. Gel and Ami.R. Gel Fast Flow are also replacing conventional **glutaraldehyde activated gels** and **cyanogen bromide activated gels** thanks to their extreme stability and cleanability (see below). These gels are now in use at major pharmaceutical companies for large-scale production of therapeutic proteins. The Ami.R. Gel Fast Flow with its outstanding high flow characteristics is optimal for use on large columns.

Directions for use

Coupling procedure

This is a recommended standard protocole given for coupling efficiently antibodies to Ami.R.Gel.
Optimization of conditions (pH, temperature, concentration) may be required depending on the Ab nature (see below).

1- Prepare the coupling buffer : Borate 0.1 M pH 9.0.
Note : Other buffers may be used within pH range of 3-10, notably phosphate for pH5-8 (also hepes, citrate, acetate…)

2- Prepare the desired quantity of Ami.R.Gel for coupling : remove the excess of solution, wash the gel on a Buchner and equilibrate with 3 ml of coupling buffer.

3- Prepare in coupling buffer 1-5 mg of antibody per ml of Ami.R.Gel.
Rem. : This quantity may be increased up to 10 mg for higher substitution, but coupling yield may be lowered.
Buffer exchange may be done by dialysis (CelluSep) or gel filtration. Pay attention to remove any ammonium salt, free amine and Tris that may have been used for antibody purification, storage, or as preservative in dialysis membranes (wash well before use).

4- Add the antibody to the gel, then add the reductant solution (1 M $NaCNBH_3$) or directly the powder to a final concentration of 0.1 M (0.2 ml/ml of gel). Incubate under constant agitation for 2 hours at room temperature.
Note : this conjugation step can be performed at +4°C for thermo-sensitive antibodies, without significant loss of yield.

5- Check the conjugation by measuring the ligand concentration in the supernatant.
The supernatant of coupling mixture may be withdrawn after decantation, and filtered to remove residual gel.
The protein concentration may be approximated by optical absorbance at 280 nm, or measuring by BC Assay (UP40840) or Coo Assay (UPF8640) methods.
If less than 10% of ligand is present in solution, continue the coupling for another 2hours.

6- Filter the gel on a Buchner, wash the resin with 10 volumes of 0.5 M NaCl.
Measure the ligand concentration-quantity in washes and initial incubated solution, then calculate the coupling yield.

7- The ligand-grafted gel can be stored at +4°C in PBS (NaCl 150 mM, phosphate 20 mM, pH7.5) + 0.5% azide, or in other suitable buffers.

*The optimal ligand substitution depends on the ligand and the protein to be purified, up 50-60mg/ml of gel for specific applications. Complex protein solutions like sera may be incubated at 5-20mg/ml of gel, while small ligands (notably peptides) may be incubated with 1-50 µmol / ml of gel.
*The pH of coupling may be choosed in the range of 3-10 and temperature in the range of 0-40°C. The coupling yield seems improved by coupling at a higher pH 9-10, and especially for monoclonal antibodies, at pH other than their isoelectric point (pI, that ranges from 5 to 8.5). The stability of the ligand to be coupled should also be considered when choosing pH.
*In normal use conditions, no endcapping is required, preserving the biological activity of the ligand.
If pH below 3.5 or above 9.5 was wished for specific applications, deactivation of the unreacted aldehyde groups may be performed by incubating with Tris 0.5 M or 0.1 M Ethanolamine + 0.1 M $NaCNBH_3$ at the same pH as the coupling buffer for 2 additional hours.
*Technical notice NT-56408.
*The coupling may also be performed in column. Incubation duration should be increased, and close circulation used.

Purification procedure with ligand-Ami.R.Gel

The use of the ligand-gel depends on your application. Often, antibody-gel are used for affinity purification, immunoprecipitation, or affinity depletion. Here are some general guidelines for the affinity purification of antigens with antibody-gel in columns :
-The gel is packed in polyethylene columns with a 40 µm frit.
-The column should be equilibrated with suitable buffer, for example PBS.
-The sample should be applied after filtration, and eventual dialysis in PBS.
-Incubate in column for >1h, or in close circulation for volume above the gel bed volume.
-Wash the column with PBS until OD280 nm is below 0.05.

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-Elute bounded antigen with citric acid 0.1 M pH3, or glycine 0.1 M, or acetate 0.1 M.
-Neutralize eluted fractions with Tris 1 M pH9.0.
-Wash the column with 10 gel volumes of PBS.

Cleaning and sanitization procedures
Autoclaving sterilization and sanitization may be performed before coupling of protein ligand to the gel, then coupling can be performed under sterile conditions.
-Suspend the gel in 100 mM NaHSO3, incubate 10 min, add 0.3 M NaOH, incubate at room temperature for 16H.
-Wash thoroughly with distilled water.

Similar procedures may be performed after ligand coupling if the ligand is stable in these conditions.

Scientific and Technical Information

A very fast, efficient, stable and reproducible coupling

- Our monoaldehyde coupling chemistry is designed for primary amines of peptides and proteins, (notably antibodies). It provides functional advantages over glutaraldehyde coupling. Glutaraldehyde is polymeric, which causes a strong Schiff-base to form that cannot be reduced completely to a secondary amine with mild reducing agents. This leaves unstable Schiff-bases remaining after the coupling takes place. These Schiff-bases slowly dissociate, releasing the bound ligand. In comparison, Schiff-bases formed with our monomeric aldehydes can be fully converted to secondary amine with mild reducing agents, eliminating the unwanted leaching of ligand from unstable linkages. Glutaraldehyde also crosslinks proteins, which reduces their activity ; monoaldehyde groups do not crosslink proteins, so activity is preserved.

Matrix-CHO + H_2N-Protein < ---> intermediate SB --->Matrix-CH_2-NH-Protein

During coupling, an instantaneous, reversible Schiff-base reaction takes place. The Schiff-base is then reduced to a stable secondary amine by the addition of a reducing agent so mild that it cannot reduce intramolecular disulfide bridges. The reduction step typically takes 20 min to 2 hours.

- Coupling can also be carried out in a packed column without agitation. This simplifies the procedure, particularly in large columns. Refrigeration during coupling is a function of the ligand, since Ami.R.Gel is stable at room temperature.
- Using Uptima Ami.R Gels, coupling efficiency is simply a function of loading. No hydrolysis or inactivation of active groups takes place during the coupling reaction, at the opposite of drawbacks that are frequently encountered with traditional coupling chemistries. This provides a highly reproducible ligands coupling, critical for both research and manufacturing. Analysis of different lots shows that ligand coupling variation is less than 5%.
- An other advantage of Ami.R Gel coupling is the fact there is no need to block remaining unreactive groups (endcapping).

Unsurpassed benefits for coupling biomolecules for biotechnological applications

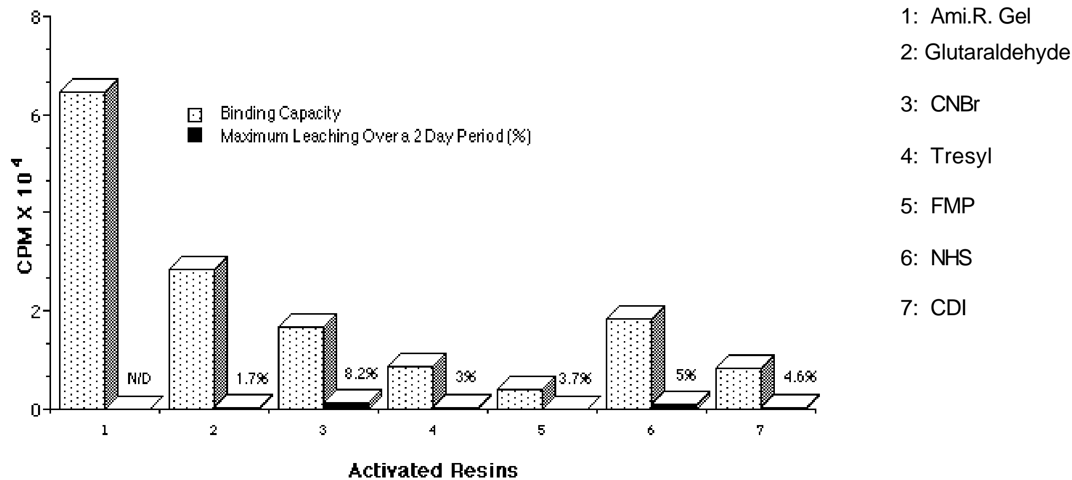
- **High loading capacity**

Protein	Loading (mg-ml resin)	Coupling Efficiency (%)
Mouse IgG	10	92
Mouse IgM	1	95
Rabbit IgG	10	94
Human IgG	10	98
Rat IgG	10	91
BSA	10	87
BSA	100	60
Bovine IgG	100	48
Pepsin	5	94
Fibrinogen	20	93

- Uptima Ami.R.Gel couples **both acidic and basic proteins** with the same high efficiency, typically more than 90% due to the high density of stable aldehyde groups.
- Uptima Ami.R.Gel has a very high activated group concentration (40-50 µmol/ml), and thus a very high protein-binding capacity. Protein coupling up to 60 mg per ml gel can be readily achieved. Up 40-50 µmoles of small ligand can be immobilized, three times the 10-15 µmoles per ml of gel available with other coupling chemistries. **Such a high binding capacity** is particularly valuable for diagnostic and production applications, when small ligands or peptides are immobilized to purify specific proteins-antibodies, to allow bioseparations of high quantities per run and at high concentrations.

- A significant problem with traditional immobilization methods is linkages of different stability forms between the gel and various nucleophilic groups (amine, thiol, hydroxyl or carboxyl) on the protein

ligand. This leads to various degrees of ligand leaching from the resin. Uptima Ami.R.Gel overcomes this problem thanks to its exclusively uniform, stable secondary amine linkages. **No leaching** of the ligand is detectable (validated as low as 0.1 ppm level because of detection sensitivity limit). As the leaving group during coupling is water, no release toxic leaving groups is observed. These features are particular issues in the manufacture of diagnostics and a crucial one in therapeutics.



1: Ami.R. Gel
2: Glutaraldehyde
3: CNBr
4: Tresyl
5: FMP
6: NHS
7: CDI

- Reaction pH significantly affects the folding and biological activity of proteins. Immobilizing proteins to their optimal pH produces the highest biological activities and binding capacities, as well as greater resistance to proteolytic degradation relative to those immobilized at non-physiological pH. Ami.R.Gel is the only way which allows you to select the physiological coupling conditions for each protein, to preserve the **maximal activity of the ligand**. Coupling can be performed at any pH between 3 and 10, whatever is optimal for maintaining the highest biological activity. Here is a comparison of the biological activity of selected proteins immobilized by different chemistries.

Immobilized	Substitution (mg-ml)	Uptima Ami.R.Gel	CNBr	Glutarald	Hydrazide
Anti-Human IgG mg of human IgG bound per mg Ab immobilized	1	0.7	0.38	0.25	0.27
Avidin % protein activity	1	100	70	-	-
Pepsin % enzyme activity	3	80	-	40	-

-Pepsin retains high biological activity when immobilized to Ami.R. Gel since coupling can be carried out at a low pH, which preserves enzymatic activity.

-Avidin is coupled to Ami.R. Gel with no loss of biotin binding activity. In contrast, CNBr coupling markedly decreases the activity of this very stable protein.

-The binding capacity of immobilized anti-human IgG for human IgG is twice as high as with CNBr, and three times that of glutaraldehyde or the "oriented" coupling (hydrazide) chemistry. IgG retains the highest biological activity, even higher than that of "oriented" coupling with hydrazide. IgG binds to AmiRGel preferably through its Fc portion. The mild monoaldehyde chemistry couples primarily through amino groups, which are the most accessible on the surface of the molecule. Therefore, the molecular structure of the proteins is only minimally affected. The "oriented" coupling immobilizes IgG through a modification of the carbohydrate moiety. It is believed that the antibody carbohydrate moiety is located primarily on the Fc portion. According to recent studies, a large percentage of the antibodies glycosylation is on the light chain (F(ab) portion), rather than on the Fc portion. The reactive aldehyde groups formed on the carbohydrate moiety may cause antibodies to polymerize due to their high concentrations of amino groups. The biological activity of the antibody after glutaraldehyde coupling and "oriented" coupling are similar, suggesting that "oriented" coupling may also cause polymerization of the antibody. Since monoaldehyde coupling chemistry binds antibodies at a preferred position on the Fc region without polymerization, the result is a binding capacity nearly three times higher.

Excellent matrix features

- **Biocompatibility :** The matrix is a high quality agarose. This gel is hydrophilic, providing very low unspecific binding with biologic samples. That makes perfect.
- **High flow rate for quick and scalled-up separations**

The efficiency and throughput of affinity separations can be improved greatly combining high binding capacity with fast flow supports. Capture flow rates up to 1,500 cm/h can be achieved in immunoaffinity chromatography with Ami.R.Gel Fast Flow. This confers **faster processing times** or smaller columns for processing the same volume of product, making it particularly useful for **process-scale applications**.

- **Easy Cleaning with NaOH, Autoclaving**

One drawback of conventional affinity supports is that they cannot be treated with sodium hydroxide, the standard cleaning method for ion exchange and gel filtration media. However, Uptima Ami.R. Gels can be depyrogenated by standard NaOH treatment. Moreover, since these media are stable at high temperature, autoclaving sterilization is also possible. These treatments have no effect on the mechanical properties or on the resins binding capacity. Once the ligand is coupled, the stability of the ligand determines the cleaning method that can be used. For example, heparin coupled to CNBr activated resin is not compatible with NaOH ; when coupled to Uptima gel, it can be cleaned and stored in NaOH.

Other Information

For any information, please contact Uptima – Interchim:
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Ordering information

	Ami.R Gel	Ami.R. Gel Fast flow
cat. number -qty:	UP56408A, 5ml	UPR2289A, 5ml

available in bulk pack sizes for industrial applications. Please inquire for pricing information.

Literature

1. Grandics, P. et al. 1990 Ann. N.Y. Acad. Sci. 589:148-156
2. Thalley, B. and Carroll, S. 1990. Bio-Technology 8:934-938
3. Schoepfer, R. et al. 1990. Neuron 5:35-48