FT-38591A

UP38591 Non-Denaturing Elution Medium

Description

Name: **Mild Elution Medium** for improved affinity purifications

Part Number : UP38591A, 1L

Storage: +4°C, DO NOT FREEZE (M)

Benefits: Elution under neutral and non-denaturing conditions
Preserves biological activity (>97%)
Increased yield (>97%)
Increases the longevity of immunoadsorbents
50-100 fold Economy per purification cycle cost
Drug Master File

Directions for use

Note: These are standard protocols for purification of antibody or antigens by affinity chromatography. Recommanded affinity support are ProteinA, G, or L gels, Ami.R.Gel (#56408) and other agarose gels.

Sample application and wash of unbound molecules

1-Apply sample to the affinity support to saturate its binding capacity. Always collect the flowthrough sample.
Note : Since the binding kinetics of antigen-antibody interaction is fast, the flow rate of sample application is determined by the flow characteristics of the gel (usually approx. 15cm/hr for 4% agarose, >3000cm/hr for 6% agarose).

2-Wash the column with 15 mM phosphate, 0.5 M NaCl, pH 7.2, until the absorbance at 280 nm is less than 0.02.

Elution

3-Elute antibodies by applying 1.5 bed volumes of gel on the column. When all of Medium entered the column bed, stop the flow for 10 min. Continue the elution with PBS (10 mM phosphate, 0.145 M NaCl, pH 7.2). Eluted material should be collected in separate vials for subsequent analysis. Usually, molecules are eluted after Mild Elution Medium passed through the 2/3 of the column (approx. 2-3 bed volumes total).
Note: Uptima Mild Elution Medium is viscous, the use of a peristaltic pump is advantageous in this step.

Calculating Recovery

Components of the Elution Medium interfere with most of the chemical protein determination methods. But one could use BC Assay #UP40840A combined to Protein Preparation Reagent #R5594A.

Protein concentration in the eluate can be estimated by spectrometry using the following formula:

$(OD_{280nm}-OD_{340nm})$ / Abs.coeff. of the eluted protein.

Notes: Spectrometric method may give biaised results because of absorbing substances in sample and co-eluted.
ODs should be corrected with ODs from the Mild Elution Buffer that has an absorption at 280nm and 340nm
The abs. coeff. for an antibody is generally: 1 mg/ml Ab solution= 1.4 OD_{280nm}.

Eluted material desalting

Equilibrate a desalting column (10x of the bed volume of the affinity column) with PBS.

Apply eluted material on the column to remove the Mild Elution Buffer.

Collect the excluded volume fraction. Concentrate antibody to 5-10 mg/ml.

Note: Removal of Medium by repeated dilution and concentration of the eluate is NOT recommended

Scientific and technical information

The high affinity of antibodies for antigens makes them difficult to elute in the solvent of application. Typically, antigens are desorbed from immunoadsorbents through the use of reagents such as chaotropic salts, acids, urea, guanidine, aliphatic acids and alcohols. However, these kinds of reagents diminish the activity of both the antigen and the immunoadsorbent, as the immunoadsorbent capacity deteriorates and eluted proteins become denatured to some degree.

- Increases Yields

Uptima Mild Elution Medium, a nondenaturing elution medium, allows quantitative recovery of antibodies from immunoadsorbents without loss of biological activity. Antigens bind with antibodies through several mechanisms including ionic and hydrophobic interactions. The formula of components break up these interactions individually. Instead of denaturing the antigen to release it from the binding, it gently desorbs antigens from the immunoadsorbent. Neutral and non-chaotropic, it contains no toxic ingredients. All components are compatible with pharmaceutical use.

- Biological Activity Retained

Retaining a protein's biological activity during purification is paramount. Typically, the protein's fragile tertiary structure becomes increasingly destabilized during lengthy, multistep procedures, causing product denaturation and significant losses in yield. Even conventional, relatively mild purification methods such as ion exchange chromatography and salt precipitation can cause product denaturation.

Affinity chromatography simplifies the purification process, rapidly achieving a high degree of product purity. However, the elution methods required can be harsh on the protein, which is a particular problem with immunoaffinity chromatography. Besides denaturing proteins, the low pH elution which is normally used can also alter critical posttranscriptional modifications such as glycosylation.

In comparison, Uptima Mild Elution Medium allows the user to elute antibodies from immunoadsorbents under neutral, protein stabilizing conditions, and delivers quantitive recovery of antibodies with no detectable loss of bioactivity:

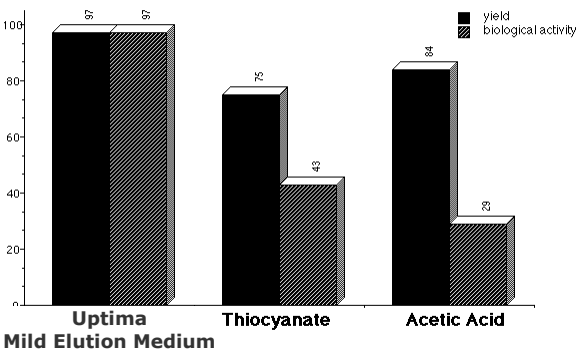
- Excellent Yield and preservation of Biological Activity

A comparison of the most commonly used acidic and chaotropic elution media was performed regarding the recovery and activity of antibodies from immunoaffinity chromatography. In parallel experiments, the recovery of bound immunoglobulin was carried out by using either Uptima Mild Elution Medium, 1M acetic acid or chaotropic (3 M NH4SCN, pH 7.0) elution media. The acetic acid eluate was neutralized prior to dialysis. Immunoreactivity was measured by ELISA and defined as OD492 units per mg of anti-IgG.

- Importantly, acidic elution not only leads to significant losses of antibody activity, but also caused subtle changes in the conformation of IgG. This is detectable by an enhanced susceptibility to peptic digestion.

- Antibodies from chaotropic thiocyanate exhibit higher bioactivity but with lower yield.

- Uptima Mild Elution Medium nearly delivers quantitive recovery of antibodies with no detectable loss of bioactivity.



The Mild Elution Buffer can be used with weak acidic conditions, it has an effective buffering range pH 4.0-7.0. Hydrochloric acid (1:1 diluted) is recommended for acidifying the medium.
Ingredients have a molecular weight less than 1000 Dalton and are nontoxic, thus are prefectly removable by conventionnal means (dialysis, ultrafiltration). This buffer is compatible with pharmacological requirements.

- Longevity of the Immunoadsorbent

The damaging effects of extreme pH on the immunoreactivity of immunoglobulin also leads to the deterioration of immunoadsorbent capacity upon continued recycling. The longevity of immunoadsorbents was examined, comparing the effects of different elution media on sorbent lifecycles.

5ml columns were packed with affinity-purified anti-rabbit IgG immobilized to Ami.R.Gel (#UP56408). Immunoadsorbents were saturated with rabbit IgG after which both bound and eluted IgG fractions were measured. Immunoadsorbents were cycled repeatedly in a continuous operation. Acidic elution medium (1 M CH3COOH, pH 2.2) was used as a control.

A characteristic "first cycle effect" was observed which is typical with immobilized polyclonal antibody columns. This leads to some loss of binding capacity during the first cycle due to extremely high affinity antigen-antibody interactions. This phenomenon is not observed with monoclonal antibody immunoadsorbents. An almost negligible 5-10% of the binding capacity of the immunoadsorbent was lost after 100 cycles using Upitma Mild Elution Medium. In comparison, control immunoadsorbent eluted with the acidic elution medium lost over 60% of its initial binding capacity within 20 cycles.

Over 500 cycles have been performed successfully on an immunoadsorbent eluted with Uptima Mild Elution Medium. However, we recommend limiting use to <100 cycles to avoid degradation of immobilized antibody, as well as the build-up of nonspecifically-adsorbed materials, such as lipids.

- Ten Times Economy

The economy of immunoaffinity purification primarily is a function of the longevity of the immunoadsorbent. Relatively, the support and ligand antibody have a small impact on process economics. Uptima Mild Elution Medium significantly improves process economy by:
- greatly extending the immunoaffinity column's useful life, over 4 fold
- improving the yield and biological activity of the purified product, generally over 4 fold
As a result, our Elution Medium reduce the overall purification cost per unit of active product by an order of magnitude (>50-100 times) below traditional immunoaffinity chromatography methods.

Other information

This product is sold for research purposes only. It is not to be used for humans or animals. There is no express or implied warranty. No liability is assumed merchantability, or direct and consequential damage. The user assumes all responsibility for care, custody and control of the material, including its disposal, in accordance with all regulations.

For any question, please **ask Uptima** or your local distributor.

Recommanded Affinity supports:

Ami.R Gel	56408
Ami.R. Gel Fast flow	R2289
Protein A affinity support	UP49981
Protein A affinity support, HC	UP90467
Protein G affinity support	UP75196

Literature

1. Grandics, P. et al. 1990 Ann. N.Y. Acad. Sci. 589:148-156
2. Thalley, B., and Carrol, S. 1990 Biotechnology 8:934-938