ZirChrom®

Technical Bulletin #243

... For Peak Performance

User Recommendations for ZirChrom Rhinophase®-AB Research and Production Kits

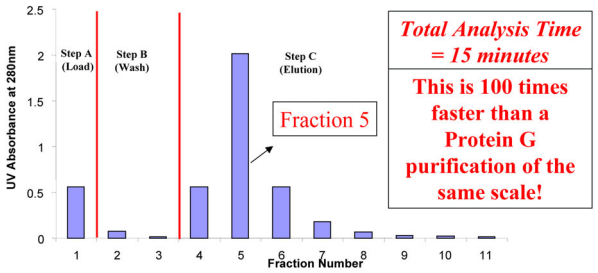
Buffer Preparation and Use

1. The purification of Monoclonal Antibodies (MABs) using the ZirChrom Rhinophase®-AB Research and Production Kits is typically a two-step process.
    a. Loading
    b. Elution

    To prepare the buffers, dissolve the contents of the respective dry buffer mix in approximately 1800 mL of HPLC grade water. Stir until all of the dry buffer mix is in solution. Using a pH meter and stirring, add a 50% Sodium Hydroxide solution until a pH of 4 is reached. Bring to a final volume of 2000 mL using desired method. It is recommended that the prepared buffers be filtered using a 0.45um membrane prior to use. Store in provided labeled containers.

2. The prepared column is initially equilibrated in Loading Buffer. Then the sample is loaded on the column and the column washed with Loading Buffer. The retained antibody is then eluted using the Elution Buffer. Typically, the first fraction will contain any contaminating proteins. Prior to the next purification, re-equilibrate the column in Loading Buffer.

Ultrafast Preparative Purification of IgG_1 Using Rhinophase®-AB



Step A = 20 mM MES buffer, 4 mM EDTPA, 50 mM NaCl @ pH 4.0, Step B = 20 mM MES buffer, 4 mM EDTPA, 50 mM NaCl @ pH 4.0, Step C = 20 mM MES buffer, 4 mM EDTPA, 2.0 M NaCl @ pH 4.0. Flow Rate = 60 mL/min, Injection size = 31.6 mL serum-free cell culture supernatant diluted 4-times with loading buffer, (3.98 mg of Mab), Amount of Rhinophase®-AB in tube = 10 grams.

Sample Preparation

The ionic strength of the sample before loading it on the column is critical in ensuring the optimum performance of the purification media. Samples originating from cell culture supernatants or other samples of high ionic strength on the order of 100-300 mM must be diluted 5-10 fold to lower the ionic strength to approximately 50 mM. In general a five-fold dilution is adequate to achieve high antibody recoveries.

Column Preparation

The ZirChrom Rhinophase®-AB product is a mechanically robust material that can withstand high mobile phase linear velocities. If the media is used in an open column format, the media should be slurried in the loading buffer and sonicated for 1 minute before pouring into the column. The second enclosed frit is to be placed on the top of the poured bed. Note: The column can be run dry without any loss of performance.

Column Storage

The ZirChrom Rhinophase®-AB product in a packed column should be stored in 50/50 2mM EDTPA in water at pH 7.0/isopropanol.

Product Information

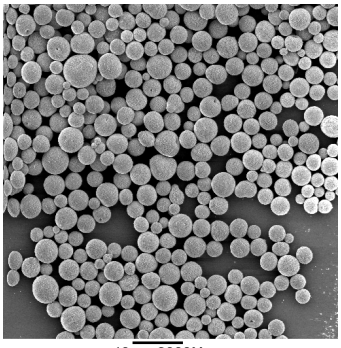
I. Analytical Grade

BET

Sample	Surface Area (m^2/g)	Pore Volume (ml/g)	Average Pore Diameter (Å)
ZirChrom Rhinophase-AB, Anal.	20	0.122	250

SEM

The final material has large pores so that large bio-molecules can diffuse into the porous beads.



II. Preparative Grade

BET

Sample	Surface Area (m^2/g)	Pore Volume (ml/g)	Average Pore Diameter (Å)
ZirChrom Rhinophase-AB, Prep.	14	0.100	300

SEM

The final material has large pores so that large bio-molecules can diffuse into the porous beads.