Quick Start Instructions for ZirChrom Rhinophase®-AB Production Kit

The ZirChrom Rhinophase®-AB Production Kit (***part# AB01-HTP***) consists of the following:

- One container containing 30 mL of Rhinophase®-AB media (25 um)
- Sixteen large (60 mL) SPE tubes with frits
- One 16-port vacuum manifold system
- One vacuum/pressure pump (110V)
- Four containers of dry loading buffer (each for 2000 mL solution)
- Four containers of dry elution buffer (each for 2000 mL solution)
- Eight empty 2000 mL plastic containers (for loading and elution buffers)

System Assembly Instructions

1. Unpack the shipping container. Set aside Rhinophase®-AB media container (smallest plastic bottle), dry loading buffer containers, dry elution buffer containers, and empty 2000 mL plastic containers.
2. Unpack the 16-port vacuum manifold. Assemble the vacuum manifold system according to the attached instructions manual. Place sample collection containers (not included) inside the manifold.
3. Unpack the vacuum/pressure pump. Install the pump according to the attached instructions manual. A section of 3/8" tubing (not included) is required to connect the vacuum/pressure pump to the vacuum manifold system.

Antibody Purification Procedure

1. Buffer Preparation: Dissolve each container of dry buffer mix (loading or elution) into approximately 1800 mL of HPLC grade water. Stir the solution until the dry buffer mix is completely dissolved. Using a pH meter and stirring, add a 50% sodium hydroxide solution until a pH of 4.0 is reached. Bring to a final volume of 2000 mL using desired method. It is recommended that each prepared buffer be filtered using a 0.45um membrane prior to use. Store each prepared buffer in provided 2000 mL container.
2. SPE Tube Packing: Insert one frit into the bottom of an SPE tube. Attach SPE tube above turn-cock valve on the top side of the vacuum manifold system. Slurry approximately 4 mL of Rhinophase®-AB media into 25 mL of prepared loading buffer and sonicate for one minute. Open turn-cock valve and pour the suspended Rhinophase®-AB media into SPE tube under vacuum. Run an additional 50 mL of loading buffer through the tube to pack bed. Insert another frit on top of the packed bed. Repeat this process to add additional SPE tubes.
3. Sample Preparation: Samples originating from cell culture supernatants or other samples of high ionic strength on the order of 100-300mM must be diluted 5-10 fold to lower the ionic strength to approximately 50mM. In general a five-fold dilution (in prepared loading buffer) is adequate to achieve high antibody recoveries.
4. Antibody Purification: The purification of monoclonal antibodies using the ZirChrom Rhinophase®-AB Production Kit is typically a two-step process.
    a. loading
    b. elution

    The packed SPE tube is initially equilibrated in approximately 25 mL of prepared loading buffer under vacuum (Note: The vacuum pressure should be set so that the flow rate is approximately 10 – 20 mL/min.). Next, load the sample into the SPE tube until the level falls below the top frit. Flush the tube with approximately 300 mL of prepared loading buffer. The retained antibody is then eluted into sample collection container(s) inside the vacuum manifold system according to the users preferred method. Typically, the first fraction will contain any contaminating proteins. Repeat this process to purify additional monoclonal antibody samples.

Product Information

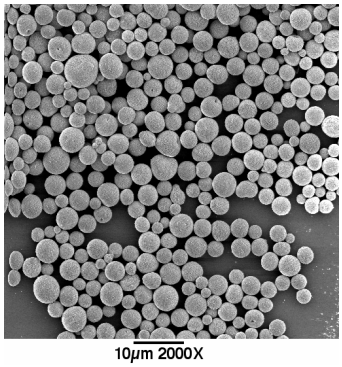
I. Analytical Grade

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.



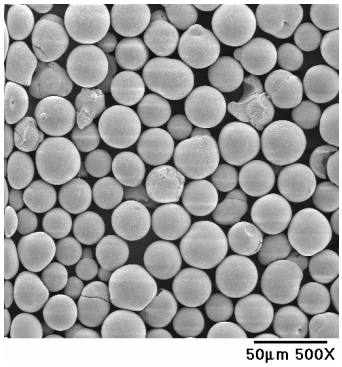
II. Preparative Grade

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.



Note: The ZirChrom Rhinophase®-AB product is a mechanically robust material that can withstand high mobile phase linear velocities. The SPE tube may be run dry without any loss of performance.