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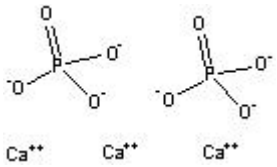
TECHNICAL INFORMATION

Catalog Number: 150162, 150163
Hydroxylapatite

Structure:



CAS #: 1306-06-4

Physical Appearance: White powder

Separation of Double- and Single-Stranded Nucleic Acids Using Hydroxylapatite Chromatography

Hydroxylapatite binds double-stranded but not single-stranded nucleic acids in lower concentrations of phosphate buffer. Double-stranded nucleic acids are eluted with a more concentrated phosphate buffer.

Basic Protocol

Materials

1. Hydroxylapatite powder
2. 0.12 M sodium phosphate buffer, pH 6.8 (0.12 M PB)
3. 0.4 M sodium phosphate buffer, pH 6.8 (0.4 M PB)
4. *sec*-butanol
5. Chloroform
6. TE buffer
7. Sephadex G-50 (Pharmacia) equilibrated in TE buffer
8. 3 M sodium acetate, pH 5.2
9. 100% and 70% ethanol
10. Silanized glass wool (see notes at end)
11. Water-jacketed column
12. Circulating pump

– Hydrate hydroxylapatite powder in 10 ml of 0.12 M PB. The hydrated volume will be ~2 to 3 ml/g powder.[1] Heat 15 to 30 min., 100ºC.
– Allow to settle at room temperature and remove buffer. Resuspend in 5 to 10 ml of 0.12 M PB at 60ºC, decant, and resuspend in 5 ml of 0.12 M PB at 60ºC.
– Pour suspension into water-jacketed column with circulating 60oC water. Wash with 5 to 10 ml of 0.12 M PB at 60ºC.[2]
– Add nucleic acid sample in 1.0 ml of 0.12 M PB at 60oC, and immediately begin collecting 1.0 ml fractions into 1.5 ml microcentrifuge tubes. Wash column 6 to 10 times with 0.5 ml of 0.12 M PB, and continue to collect fractions. Most of the single-stranded nucleic acids will elute in the first three fractions. Elute double-stranded nucleic acids by washing the column 8 times with 0.5 ml of 0.4 M PB.
– Reduce each fraction to ~200 ul by repeatedly extracting wit *sec*-butanol (the organic phase will be on top). Remove *sec* -butanol by extracting once with an equal volume of chloroform (the organic phase will be on the bottom). Transfer aqueous phase to a microcentrifuge tube in a fume hood for 30 min., room temperature, to allow the residual chloroform to evaporate.
– Bring volume to 200 ul with TE buffer. Desalt over Sephadex G-50 spin column, prepared using silanized glass wool and a 5-ml disposable syringe (see notes below).
– Add 20 ul of 3 M sodium acetate, pH 5.2 and 500 ul of 100% ethanol. Mix, then incubate 30 min. at -20ºC. Microcentrifuge 10 min. at 4ºC, rinse with 70% ethanol at 4ºC, air dry, and resuspend in TE buffer.

[1]Because of its high binding capacity (~600 ug nucleic acid/g hydroxylapatite for MP # 150163), a 0.5 ml column is sufficient.
[2]The flow rate can be increased by capping the top of the column and applying positive pressure using a syringe (for MP # 150162).

Notes

– Spin-Column

– Plug the bottom of a 5-ml disposable syringe with clean, silanized glass wool. Fill the syringe with an even suspension of the column resin, and place in a polypropylene tube. Centrifuge in a swinging bucket rotor of a tabletop centrifuge 2 to 3 minutes at a setting of 4.
– Dilute the radioactive sample with TE buffer to 100 ul and load in the center of the column. Place the syringe in a new polypropylene tube. Centrifuge 5 min. at a setting of 5 to 6.
– Save the liquid at the bottom of the tube containing the DNA. Discard the syringe, which retains the unincorporated dNTPs.

– Glassware is silanized (siliconized) to prevent adsorption of solute to the glass surface or to increase its hydrophobicity.

– In a fume hood, place in desiccator the glassware or equipment to be silanized and a beaker containing 1 to 3 ml chlorotrimethylsilane or dichlorodimethylsilane. **CAUTION**: Chlorotrimethylsilane and dichlorodimethylsilane vapors are toxic and highly flammable. Items too large to fit in desiccator can be silanized by briefly rinsing with ~5% dichlorodimethylsilane in volatile organic solvents (chloroform or heptane). Remove the organic solvent by evaporation. This method is useful for treating glass plates for denaturing polyacrylamide sequencing gels.
– Connect desiccator to vacuum pump until silane starts to boil, close connection to pump, and maintain vacuum. (During the incubation the silane will evaporate, be deposited on the surface of the glassware, and polymerize. Do not leave the desiccator attached to the pump. This will suck away the silane, minimizing deposition and damaging the pump). Leave the desiccator evacuated and closed until liquid silane is gone (~1 to 3 hours).
– Open disiccator in a fume hood for several minutes to disperse silane vapors. If desired, bake or autoclave glassware or apparatus. **CAUTION**: If a flammable solvent is used, do not bake the glassware until the solvent is completely evaporated.

– TE buffer, pH 7.4, 7.5, or 8.0
10 mM Tris, pH 7.5, 7.5, or 8.0
1 mM EDTA, pH 8.0

This procedure is from: *Short Protocols in Molecular Biology* (MP catalog # 154428), Eds: Frederick M. Ausebel, et. al. Published by: Greene Publishing Associates and Wiley-Interscience, 1989.

Protocol contributed by Eric J. Richards