

DNA Extraction of Viruses Using Wizard Columns

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Components

Wizard DNA Purification Resin (Promega #A7181)

Wizard Mini Columns (Promega #A7211)

Phage Buffer to dilute if necessary (150mM NaCl, 40mM Tris-Cl, pH7.4, 10mM MgSO₄ in nuclease-free water; fliter sterilized)

Procedure

1. Mix 1ml DNA Purification Resin with 0.5 ml CsCl sample (can use up to 1ml sample but more than that will significantly decrease yield of DNA).
 - a. Note: if you have more than 1ml of CsCl sample, you can use more Wizard columns, or you can concentrate prior to DNA extraction using Amicon Ultra Concentrators (100kDa MWCO). Try to use the size that fits most of your sample in one or two spins; spin at 1000g for 5 min at 10°C and check volume. If you need to add more volume to the retenate, use the flow through to do this.
2. Attach minicolumn to bottom of 3ml or 5ml sterile syringe that has had plunger removed.
3. Add resin with sample to the syringe and push through the solution (can save flow-thru just in case you think you overloaded the resin).
4. Remove minicolumn from the syringe and pull out plunger.
5. Reattach minicolumn to the syringe and 2ml of 80% isopropanol to the syringe.
6. Using the plunger push through the isopropanol to wash the resin.
7. Remove minicolumn from syringe and place in a sterile 1.5ml centrifuge tube.
8. Centrifuge 10,000 g for 2min to remove any residual liquid.
9. Place minicolumn in new sterile 1.5ml centrifuge tube.
10. Add 100µl 80°C TE buffer to top of minicolumn.
11. Place tube lid over top of column and vortex gently for 10 seconds.
12. Wait one minute and then centrifuge at 10,000 g for 30 sec to elute DNA.
13. Can repeat this a second time using 50µl warm TE (do not pool the 2 elutions until you quantify so as not to dilute the sample). Usually can recover an addition 10-20% of DNA with the second elution.
14. Quantify DNA by Quant-iT PicoGreen dsDNA assay kit (Invitrogen #P7589).