



Forensic Biology Section

Amicon Ultra DNA Filtration

1. **Scope**

- 1.1. The Amicon Ultra 100k centrifugal filter devices concentrate solutions of DNA through a cellulose membrane using a fixed-angle rotor centrifuge.
- 1.2. The devices are used to filter or concentrate in the following instances:
 - 1.2.1. When very low yields of DNA are obtained after the extraction procedure, the volume of the DNA extract can be reduced in order to increase the extract's concentration so more DNA can be added to the amplification reaction.
 - 1.2.2. When the presence of PCR inhibitors are suspected, the DNA extract can be filtered and then brought back up to a suitable volume for amplification in an attempt to remove the inhibitors.
 - 1.2.3. As the final clean-up step following phenol/chloroform organic extractions.

2. **Safety**

- 2.1. **WARNING: CHEMICAL HAZARD.** Wear chemical-resistant gloves and eye protection when handling phenol-chloroform solution. Use in well-ventilated area.
- 2.2. Treat all biological specimens as potentially infectious. Gloves and a laboratory coat must be worn at all times and follow Universal Precautions.

3. **Specimen**

- 3.1. Phenol/chloroform DNA extracts.
- 3.2. DNA extracts containing a low concentration of DNA.
- 3.3. DNA extracts believed to contain PCR inhibitors.

4. **Equipment**

- Fixed-angle centrifuge (e.g. Eppendorf 5415 or 5430)

5. **Procedural Notes**

- 5.1. Amicon Filter components cannot be autoclaved or irradiated.
- 5.2. A reagent blank (or portion of a reagent blank) should be carried through the filtration procedure and assayed in parallel with the evidence samples.
- 5.3. Do not touch the pipette tip to the membrane inside the Amicon column.
- 5.4. Avoid transferring any phenol/chloroform organic phase to the filter device.

6. **Filtering DNA extracts**

- 6.1. Assemble and label the filter device for each sample to be extracted (including Reagent Blanks) by inserting a "sample reservoir" into an open "retentate/filtrate vial". Close the caps on the assemblies and label the tube with case number and item number.
- 6.2. Label one additional "retentate/filtrate vial" for each sample (for recovery at end of procedure).



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- 6.3. Carefully transfer up to 500 μ l of DNA extract into the “sample reservoir” (upper chamber) of the filter device. Cap tightly with attached lid.
- 6.4. Centrifuge the tubes at least 10 minutes at **14,000x g** (~11,000 rpm in Eppendorf 5430; ~12,000 rpm in Eppendorf 5415). Repeat centrifugation step until almost all liquid is filtered through the membrane, leaving only a residue in the tip of the sample reservoir.
- 6.5. NOTE: If liquid is slow to pass through the filter, the following may have occurred:
 - 6.5.1. The filter may be clogged (in which case, the retentate should be transferred to a new filter device).
 - 6.5.2. The DNA solution may be too concentrated (in which case, the sample may be quantitated at whatever volume has been achieved).
 - 6.5.3. The sample may be too cold to pass through the filter efficiently (in which case, the sample needs to warm up to room temperature).
- 6.6. If filtering an organic extraction: discard the filtrate (liquid that has passed through the filter into the “retentate/filtrate vial”), add 500 μ l of TE⁻⁴ buffer to the “sample reservoir” and repeat the centrifugation in step 6.4 above.
- 6.7. Place sample reservoir upside down in the second labeled “retentate/filtrate vial”. Spin for 2 minutes at **1000 x g** (~3,000 rpm in Eppendorf 5430; ~2,000 rpm in Eppendorf 5415) to transfer retentate into new vial.
- 6.8. Bring retentate up to volume with TE⁻⁴ buffer if necessary, for a final volume of as little as 20 μ l, or as much as 200 μ l.
- 6.9. The sample can now be stored in a refrigerator (1° C-8° C) for short-term storage, stored in a freezer (-10° C or colder) for long-term storage, or processed in downstream applications.