# AquaPure® Genomic DNA Isolation Kit*: Frequently Asked Technical Questions

## Methodology

**On what methodology is the AquaPure genomic DNA isolation kit based?**
The AquaPure genomic DNA isolation kit is based on a modified salt-precipitation method.

**What is the expected $A_{260}/A_{280}$ ratio?**
The expected $A_{260}/A_{280}$ ratio is 1.7–2.0.

**What is the expected size of the isolated genomic DNA?**
On average, isolated DNA is >100 kb, usually 100–200 kb.

**What are the components of the DNA hydration solution?**
The hydration solution is 10 mM Tris, 1 mM EDTA, pH 7.0–8.0.

## Storage

**At what temperature should the AquaPure genomic DNA kit be stored?**
Store all solutions at room temperature except the RNase A solution, which should be stored at 4ºC.

**The cell lysis solution contains a precipitate. Can it still be used?**
The SDS in the cell lysis solution precipitates out if exposed to cool temperatures during shipping or storage. Just warm the cell lysis solution in a 37–65ºC water bath or oven until the precipitate dissolves, and mix well before use.

**How long are the AquaPure genomic DNA isolation kit solutions stable?**
All solutions are stable for at least 3 years from the date of manufacture.

**How long is the RNase A solution stable at room temperature?**
The RNase A solution is stable for at least 8 weeks at room temperature.

**Can I store the isolated DNA and use it later?**
Yes, AquaPure-isolated genomic DNA is stable for at least 5 years at 4ºC. For long-term storage, the DNA may be stored at -20ºC, but we recommend avoiding repeated freezing and thawing to reduce DNA damage.

## Sample Protocols

**For blood collection, what type of tube do you recommend?**
We recommend using EDTA (lavender top) tubes for blood collection. ACD (yellow top) tubes may also be used. However, we do not recommend using tubes containing heparin as an anticoagulant, since heparin has been shown to inhibit Taq polymerase in PCR** protocols.

**Can I isolate DNA from frozen blood samples?**
Yes. We recommend removing the blood from the freezer, thawing it quickly in a 37ºC water bath and then placing it on ice until use to reduce endogenous DNase activity. Then, proceed with the red blood cell lysis step of the DNA isolation protocol for isolating DNA from whole blood. It is helpful to freeze the blood in aliquots that will be used for the DNA isolation.

**Can I isolate DNA from clotted blood using the AquaPure genomic DNA isolation kit?**
Yes, a protocol is available for isolating DNA from clotted blood samples. Please contact Bio-Rad's technical support service for details.

**Can I repeat the red blood cell lysis step if all red cells were not completely lysed during the initial incubation?**
Yes, the red blood cell lysis step may be repeated. Incomplete lysis of red blood cells may occur when the number of red blood cells in the sample is too large.

**What should I do if there are cell clumps in my sample after adding cell lysis solution?**
Cell clumps may occur when cells are not completely resuspended prior to addition of the cell lysis solution. Incubate the sample in cell lysis solution at 37ºC or room temperature with periodic mixing until the solution is homogeneous. Or, add proteinase K to the sample (final concentration of 100 µg/ml) and incubate at 55ºC until cells are completely lysed.

* Catalog #732-6340

** The polymerase chain reaction (PCR) process is covered by patents owned by Hoffman-LaRoche. Use of the PCRprocess requires a license.

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**What if I do not see a protein pellet or if the protein pellet is loose after the protein precipitation step?**
We recommend vortexing the sample again for 20 sec and then placing it on ice for 5 min before centrifuging to pellet the proteins. Loose protein pellets can occur if the sample was not cooled to room temperature before adding the protein precipitation solution or if the sample was not vortexed for the full 20 sec to mix the solution uniformly with the cell lysate.

**Does vortexing the sample for 20 sec at the protein precipitation step shear the DNA?**
No, the AquaPure genomic DNA method is a very gentle modified salt-precipitation procedure. Vortexing with this method minimizes shearing compared to vortexing when using organic-solvent or column-based methods, which utilize harsh chemicals.

**Is it possible to isolate DNA from a smaller or larger sample size than described in the protocol booklet?**
Yes, because the AquaPure genomic DNA kit is a reagent-based system, the protocols are easily scaled up or down depending on sample size.

**Can I "speed-vac" the samples to dry the DNA?**
No, we do not recommend drying the DNA in this manner since the DNA samples will be easily overdried, making them difficult to rehydrate. We recommend allowing the DNA to air-dry for 10–15 min to evaporate the ethanol. Any droplets remaining in the tube will contain only water, which will not interfere with subsequent analysis of the DNA.

**How should I determine the DNA yield of my samples?**
We recommend quantitating DNA by UV spectrophotometry.

**The $A_{260}/A_{280}$ ratio is <1.6, indicating protein contamination of my DNA samples. How can I clean up the DNA?**
The DNA may be reisolated to remove protein contamination, which is usually caused by exceeding the recommended amount of starting sample. It is important to use the amount of starting sample material specified in the protocol.

**What should I do when the $A_{260}/A_{280}$ ratio is >2.0, indicating RNA contamination?**
Repeat the RNase A treatment and reprecipitate the DNA. In samples that contain large amounts of RNA, it may be necessary to increase the RNase A treatment time from 15 min to 30–60 min to completely digest the RNA.

**What are good stopping points when isolating DNA using the AquaPure genomic DNA isolation kit?**
Good stopping points in the AquaPure genomic DNA isolation kit protocols are:

- After adding cell lysis solution; most samples are stable for at least 18 months at room temperature
- After addition of 100% isopropanol (2-propanol); samples may be stored indefinitely at room temperature

**Why did I obtain a lower than expected DNA yield?**
The yield may be lower than expected if cells are not completely lysed. It is very important to use the amount of starting material specified in the protocol. Too few cells may create an imbalance in the DNA isolation chemistry and inhibit DNA precipitation. Too many cells may overload the system, inhibiting complete cell lysis. In either situation, the result is a low yield of DNA.

**My sample contains a low cell number and I expect a low yield of DNA; how can I obtain the maximum yield?**
When isolating DNA from samples containing fewer than 200,000 cells, or when a low yield is expected, glycogen may be added to the isopropanol to act as a DNA carrier. We recommend adding 1 µl glycogen solution (20 mg/ml) per 600 µl isopropanol.

## Applications

**Is the DNA clean enough to do PCR? Southerns? Sequencing?**
Yes! Yes! Yes!

**Why wasn't my DNA amplified using PCR?**
This method of DNA isolation often produces very high yields. When too much DNA is added to a PCR, the reaction is inhibited. We recommend adding 25–1,000 ng DNA per 50 µl reaction, with the DNA in a volume of ≤5 µl. Above that, the $MgCl_2$ concentration in the PCR may have to be increased.

**Will I have to increase the $MgCl_2$ concentration in my PCR since the DNA hydration solution contains EDTA?**
We recommend adding a DNA volume of up to 1/10 the volume of the PCR. Above that, the $MgCl_2$ concentration in the PCR may have to be increased.

**Why wasn't my DNA completely digested with restriction enzymes?**
A common cause of incomplete digestion is adding too much DNA to the reaction. We recommend digesting DNA using 2 units of enzyme per µg of DNA.