

Apoptotic DNA Ladder Kit Protocol

Important Notes

Please read the following notes before starting of the Apoptotic DNA Ladder Kit procedures.

Before equipment

- **Buffer NL:** shaking before use.
Note: If a precipitate has formed, dissolve by incubating at 56°C. Do not add Proteinase K directly to Buffer NL.
- **Add ethanol (96-100%) to Wash D Solution**, before use first (see bottle label for volume).
- Warm the Elution Buffer to 70°C, all other reagents should be at 15-25°C.
- The binding buffer (vial 1) contains guanidine-HCl which is an irritant. Wear gloves and follow

1. **Pipet 20 ul Proteinase K into the bottom of a 1.5 ml microcentrifuge tube.**
2. **Add 2 x 10⁶ cells in a sample volume of 200 ul to the microcentrifuge tube. (200 ul whole blood contains about 2 x 10⁶ cells; add PBS to the sample up to 200 ul if necessary).**
Spin columns copurify RNA and DNA when both are present in the sample. RNA may inhibit some downstream enzymatic reactions, but not PCR. If RNA-free genomic DNA is required, 4 ul of an RNase A stock solution (100 mg/ml) should be added to the sample before addition of Buffer NL.
3. **Add 200 ul Buffer NL to the sample. Mix by pulse-vortexing for 15 sec.**
If the sample volume is larger than 200 ul, increase the amount of Proteinase K and Buffer NL proportionally; e.g., a 400 ul sample will require 40 ul Proteinase K and 400 ul Buffer NL.
Note: Do not add Proteinase K directly to Buffer NL.
4. **Incubate at 56°C for 10 min.**
Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
5. **Add 100 ul isopropanol to the sample, and mix again by pulse-vortexing for 15 s.**
After mixing, briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from the inside of the lid.
If the sample volume is greater than 400 ul, increase the amount of alcohol proportionally; e.g., a 400 ul sample will require 800 ul of alcohol.
6. **Carefully apply the mixture from step 5 to the Spin Column (in a 2 ml collection tube) without wetting the rim, close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min.** Place the spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.
If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the spin column is empty.
7. **Carefully open the spin column and add 500 ul Wash D Solution without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min.** Place the spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.
It is not necessary to increase the volume of Wash C Solution if the original sample volume is larger than 200 ul.
8. **Carefully open the spin column and add 500 ul Wash D Solution without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Finally centrifuge for 10 s at max. speed (13 000 rpm) to remove residual wash buffer.**
9. **Place the spin column in a clean 1.5 ml microcentrifuge tube, and discard the collection tube containing the filtrate. Carefully open the spin column and add 200 ul of prewarmed (70°C) Elution Buffer or distilled water. Incubate at room temperature (15-25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.**
Incubating the spin column loaded with Elution Buffer or water for 5 min at room temperature before centrifugation generally increases DNA yield.
A second elution step with a further 200 ul Elution Buffer will increase yields by up to 15%.
Volumes of more than 200 ul should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation. Elution with volumes of less than 200 ul increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. For samples containing less than 1 ug of DNA, elution in 50 ul Elution Buffer or water is recommended. Eluting with 2 x 100 ul instead of 1 x 200 ul does not increase elution efficiency.
Note: The DNA is stable and can be used directly or stored at -20 °C for later analysis (do not store longer than 14 days).