prepIT®•L2P

Laboratory protocol for manual purification of DNA from 0.5 mL using a deep 96-well plate

Ethanol precipitation protocol and prepIT®•L2P reagent for the purification of genomic DNA from the Oragene® and ORAcollect® families of collection kits.

Visit our website at www.dnagenotek.com for any additional languages and protocols.

The following step-by-step protocol describes how to purify 500 µL aliquots of multiple samples simultaneously using a deep 96-well plate.

Reagents included

- prepIT®L2P (catalog #: PT-L2P)

Equipment and reagents

- Plates and covers - deep 96-well (round) plates (e.g., Axygen Cat. No. P-DW-20-C) with 96-well reusable mat or adhesive cover sheet (e.g., Axygen Cat. No. AM-2ML-RD-IMP)
- Centrifuge with bucket to accommodate 96-well plates. Capable of a minimum of 3,500 x *g*. (e.g., Sorvall centrifuge model RT 6000D with PN 11093 96-well plate adapter)
- -20°C freezer
- Blue Dextran (1 mg/mL) (Sigma-Aldrich Cat. No. D5751)
- Isopropanol at room temperature
- 70% ethanol at room temperature
- DNA storage buffer: TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- 8- or 12-channel pipettor (optional)

Procedure

Purification steps	Notes
1. Mix the sample in the DNA Genotek kit by inversion and gentle shaking for a few seconds.	• This is to ensure that viscous samples are properly mixed.
2. Incubate the sample at 50°C in a water incubator for a minimum of 1 hour or in an air incubator for a minimum of 2 hours. **Note:** The use of an air incubator may be preferable since the sample tubes may float in a water bath. If a water bath must be used, ensure the sample-containing portion of the tube remains immersed in water.	• This heat-treatment step is essential to ensure that DNA is adequately released and that nucleases are permanently inactivated. • This incubation step may be performed at any time after sample is collected and before it is purified. • The entire sample must be incubated in the original collection tube before aliquoting to ensure sample homogeneity. • The sample may be incubated at 50°C overnight if it is more convenient. • A longer time is required in an air incubator because temperature equilibration is slower than in a water incubator.
3. Add 20 µL of PT-L2P to each well of the plate.	
4. Transfer 5 µL of Blue Dextran (1 mg/mL) to each well of the plate.	• Blue Dextran helps to make the DNA pellet more visible during the DNA precipitation step.

Purification steps	Notes
5. Transfer 500 µL of sample to each well.	
6. Cover plate with adhesive cover sheet or reusable mat. Press into place to seal. Mix manually by inversion 5 times.	• Ensure a firm seal for each well. • The sample will become turbid as impurities and inhibitors are precipitated.
7. Incubate at -20°C for 10 minutes.	
8. Centrifuge plate at room temperature for 10 minutes at 4,200 x *g*.	• It is recommended that all centrifugation steps in the protocol be performed at 4,200 x *g*. However, if centrifuge is incapable of 4,200 x *g* a minimum of 3,500 x *g* is acceptable.
9. During centrifugation, label a second 96-well plate.	
10. When centrifuge has stopped, transfer 450 µL of the supernatant from the first plate to the second plate. **Take care not to disrupt the pellet.**	• The pellet contains turbid impurities. Discard plate containing pellets once supernatant is transferred.
11. To the new plate containing supernatant add 350 µL of Isopropanol (room temperature) to each well.	
12. Cover plate with adhesive cover sheet or reusable mat. Press into place to seal. Mix manually by slowly inverting 10 times. Incubate at room temperature for 10 minutes.	• During mixing with isopropanol, the DNA will be precipitated. This may appear as a clot of DNA fibers or as a fine precipitate, depending upon the amount of DNA in the sample. • Even if no clot is seen, DNA will be recovered by carefully following the next steps.
13. Centrifuge plate at room temperature for 10 minutes at 4,200 x *g*.	
14. Carefully remove as much supernatant as possible from each well without disturbing the pellet. Discard the supernatant.	• This step should be done with a single-tip pipette to avoid disturbing the pellet. Some pellets may be stuck to the side of the well, take care not to remove them with the pipette. • This pellet contains DNA. Loss of the pellet will result in loss of DNA.
15. Add 400 µL of 70% ethanol (room temperature) to each well.	• Take care not to disturb the DNA pellet. • The DNA pellet may be small. • The 70% ethanol wash helps to remove residual inhibitors.
16. Cover plate with adhesive cover sheet or reusable mat. Press into place to seal. Mix by vigorous vortexing.	

Purification steps	Notes
17. Centrifuge plate at room temperature for 10 minutes at 4,200 x *g*.	
18. **Carefully remove ALL of the supernatant from each well without disturbing the pellet.** Discard the supernatant.	• To avoid disturbing the pellet the use of a single-channel pipette is recommended for this step. • **It is important to remove all ethanol from the sample. Carryover of ethanol may impact the performance of the assay.**
19. The plate should be pulsed centrifuged for 20 seconds to collect any left over ethanol.	
20. Using a pipette carefully remove **ALL** residual ethanol and air-dry the plate for 5 minutes.	• To avoid disturbing the pellet the use of a single-channel pipette is recommended for this step.
21. Add 50-100 µL of TE buffer to each well and cover plate with adhesive cover sheet or reusable mat.	
22. Vortex vigorously to ensure any pellet on the side of the well is dislodged and re-hydrated. Place the plate on a shaker or rocker to help fully re-hydrate the DNA pellet overnight.	• To increase the rate of DNA hydration and maximize recovery the sample may be incubated for 1 hour at 50°C with occasional vortexing.
23. Options for storage of the fully rehydrated DNA: a) **Recommended** in TE, in aliquots at -20°C for long-term storage, or b) In TE at 4°C for up to 2 months.	• Freezing of purified DNA in TE will cause DNA to precipitate. When thawing a sample of frozen purified DNA follow the heating instructions from the note in step 22 to ensure complete re-hydration.

Quantification of DNA

By fluorescence method

Assays that use fluorescent dyes are more specific than absorbance at 260 nm for quantifying the amount of double-stranded DNA (dsDNA) in a DNA sample. We recommend using fluorescent dyes such as PicoGreen® or SYBR® Green I to quantify dsDNA since there is less interference by contaminating RNA. An inexpensive protocol using SYBR Green I is described in PD-PR-075, *DNA quantification using SYBR Green I Dye and a micro-plate reader*[1]. Alternatively, commercially available kits such as Invitrogen's Quant-iT™ PicoGreen dsDNA Assay Kit (Cat. No. Q-33130) can be used. For either protocol, we recommend that the purified DNA be diluted 1:50 with TE solution and that 5 µL be used in the quantification assay.

By absorbance method

If you choose to quantify DNA by absorbance, we recommend that you first treat the purified sample with RNase to digest contaminating RNA and then remove the RNA fragments by ethanol precipitation of the DNA. A detailed protocol is described in PD-PR-040, *RNA removal by double-RNase digestion*[2]. Please note that DNA from an oral sample typically contains appreciably more RNA than found in blood samples. Ensure that alcohol-precipitated DNA is fully dissolved before reading the absorbance.

Conversion factor: An absorbance of 1.0 at 260 nm corresponds to a concentration of 50 ng/µL (50 µg/mL) for pure dsDNA.

Ensure that absorbance values are within the linear range of the spectrophotometer. Re-dilute and re-measure samples that fall outside of the linear range. See your instrument documentation for more information.

Method:

1. Dilute a 10 µL aliquot of purified RNase-treated DNA with 90 µL of TE (1/10 dilution). Mix by gently pipetting up and down. Wait for bubbles to clear.
2. Use TE in the reference (blank) cell.
3. Measure absorbance at 320 nm, 280 nm and 260 nm.
4. Calculate corrected A_{280} and A_{260} values by subtracting the absorbance at 320 nm (A_{320}) from the A_{280} and A_{260} values.
5. DNA concentration in ng/µL = corrected $A_{260} \times 10$ (dilution factor) $\times 50$ (conversion factor).
6. A_{260}/A_{280} ratio: Divide corrected A_{260} by corrected A_{280}.

Example

1. Assume the measured A_{320}= 0.025, A_{280}= 0.175 and A_{260}= 0.295
2. The DNA concentration of the undiluted sample will be:
   $(A_{260} - A_{320}) \times 10$ [dilution factor] $\times 50$ [conversion factor]
   $= (0.295 - 0.025) \times 10 \times 50$
   $= 0.270 \times 10 \times 50$
   = 135 ng/µL or 135 µg/mL
3. The corrected A_{260}/A_{280} ratio will be:
   $(A_{260} - A_{320}) \div (A_{280} - A_{320})$
   $= (0.296 - 0.025) \div (0.175 - 0.025)$
   $= 0.270 \div 0.150$
   = 1.80

References

[1] DNA quantification using the Fluorescence/DNase (F/D) assay. Replaced by DNA quantification using SYBR Green I dye and a micro-plate reader. DNA Genotek. PD-PR-075.

[2] RNA removal by double-RNase digestion. DNA Genotek. PD-PR-040.

Technical support is available Monday to Friday (9h00 to 17h00 EST):

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: 613.723.5757, option 6
- Email: support@dnagenotek.com