Manual purification protocol handbook

for use with

prepIT™•L2P

DNAgenotek™

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Superior samples
Proven performance

CE IVD

The prepIT™•L2P protocol is available in additional languages at www.dnagenotek.com

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Intended use/purpose
For the purification of genomic DNA from the Oragene™ and ORAcollect™ saliva collection kits.

In-use stability
PT-L2P-5 (5 mL) and PT-L2P-45 (45 mL) have 30 months of in-use stability at room temperature.

Features
- Optimized chemistry for maximum recovery of DNA from oral samples collected with Oragene and ORAcollect product lines.
- Proven to provide consistent results with high-molecular weight DNA.
- Scalable purification method for large or small sample volumes.
- Convenient workflow with complete technical support from collection through extraction.
- Cost effective method that requires minimal equipment.

Materials
- PT-L2P-5 (5 mL) and/or PT-L2P-45 (45 mL)
- prepIT•L2P product handbook

Warning and precautions
- For Laboratory Use only.
- Do NOT ingest liquid reagent.
- Do NOT use if packaging is damaged or seal in the funnel lid/cap is broken or leaking.
- Do NOT use prepIT•L2P beyond the "Use by" date indicated on the reagent bottle.
- Wash with water if reagent comes in contact with eyes or skin. Do NOT ingest.
- Report any serious incident to DNA Genotek and the competent authority in your country.
- Refer to MSDS for safe disposal of unused reagent.
- Material Safety Data Sheet (MSDS) is available at www.dnagenotek.com.

Product use limitations
Use prepIT•L2P only as directed in this product handbook.

Transportation of prepIT•L2P
prepIT•L2P can be transported at room temperature as a laboratory reagent. No special handling is required.

Storage of prepIT•L2P (Shelf-life)
Store at room temperature. Shelf-life for PT-L2P-5 (5mL) and PT-L2P-45 (45 mL) shall be 30 months when properly capped and stored at room temperature.

Disposal
Discard unused, damaged or leaking kits in accordance with appropriate local, state and federal regulations. Discard as laboratory waste.

Maintenance/repairs
Not applicable. prepIT•L2P is a reagent — no maintenance or repair required.

Summary of performance characteristics
prepIT•L2P purified genomic DNA from Oragene™ and ORAcollect™ saliva collection kits provides high quality and quantity DNA sufficient for use in downstream applications, such as PCR, microarray and next generation sequencing.

Product presentations
prepIT•L2P is available in multiple volumes, depending on the number of preparations required. For example:

Product reference/ Catalog number	Sample preparation volume	Number of preparations
PT-L2P-5	0.5 mL	200
PT-L2P-45	0.5 mL	2,000

Warranties
Full terms and conditions for all DNA Genotek products are at http://www.dnagenotek.com/ROW/terms/index.html.

Troubleshooting
Contact DNA Genotek technical support at support@dnagenotek.com or call +1 (613) 723-5757, option 6.

prepIT™ laboratory protocol
for manual purification of DNA from 500 µL of sample

The following step-by-step protocol describes how to purify DNA from a 500 µL aliquot of sample.

Reagents included
prepIT•L2P (Cat. No. PT-L2P-5 or PT-L2P-45)

Equipment and reagents
- Microcentrifuge capable of running at 15,000 × *g*
- 1.5 mL microtubes (e.g., Axygen® Cat. No. MCT-150-C)
- Air or water incubator at 50°C
- Ethanol (95% to 100%) at room temperature
- Ethanol (70%) at room temperature
- DNA storage buffer: TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or similar solution

Procedure

Purification steps	Notes
1. Mix the Oragene/ORAcollect sample by inversion or gentle shaking for a few seconds.	• This is to ensure that viscous samples are properly mixed.

Purification steps	Notes
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.	• 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. **Note:** The use of an air incubator may be preferable since the Oragene/ORAcollect 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.
3. Transfer 500 µL of the mixed sample to a 1.5 mL microcentrifuge tube.	• The remainder of the sample can be stored at room temperature (15°C to 25°C) or frozen. • If desired, the sample may be stored frozen in the Oragene/ORAcollect tube at -20°C or the sample may be transferred to a cryovial for long-term storage at -80°C.
4. Add 20 µL (1/25th volume) of prepIT•L2P to the microcentrifuge tube and mix by vortexing for a few seconds.	• The sample will become turbid as impurities and inhibitors are precipitated.
5. Incubate on ice for 10 minutes.	• Room temperature incubation can be substituted but will be slightly less effective in removing impurities.

Purification steps	Notes
6. Centrifuge at room temperature for 5 minutes at 15,000 × *g*.	• A longer period of centrifugation (up to 15 minutes) may be beneficial in reducing the turbidity (high A_{320}) of the final DNA solution.
7. Carefully transfer the clear supernatant with a pipette tip into a fresh microcentrifuge tube. **Discard the pellet containing impurities.**	• The pellet contains turbid impurities. If accidentally disturbed, the tube should be re-centrifuged.
8. Add 600 µL of room temperature 95% to 100% ethanol. Mix gently by inversion 10 times.	• During mixing with ethanol, the DNA will be precipitated. This may appear as a clot of DNA fibres 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.
9. Allow the sample to stand at room temperature for 10 minutes to allow the DNA to fully precipitate.	• Incubation at -20°C is not recommended because impurities may co-precipitate with the DNA.
10. Place the tube in the microcentrifuge in a known orientation. Centrifuge at room temperature for 2 minutes at 15,000 × *g*.	• For example, place each tube in the microcentrifuge, with the hinge portion of the cap pointing away from the centre of the rotor. After centrifugation, the position of the pellet can be located (even if too tiny to be visible); it will be at the tip of the tube below the hinge.
11. Carefully remove the supernatant with a pipette tip and discard it. Take care to avoid disturbing the DNA pellet.	• This pellet contains DNA. Loss of the pellet will result in loss of the DNA. • Rotating the tube such that the pellet is on the upper wall will allow you to safely move a pipette tip along the lower wall and remove all of the supernatant. • The supernatant may contain impurities and should be removed as completely as possible.

Purification steps	Notes
12. Ethanol wash: Carefully add 250 µL of 70% ethanol. Let stand at room temperature for 1 minute. **Completely remove the ethanol without disturbing the pellet.**	• **It is important to remove all ethanol from the sample. Carry-over of ethanol may impact the performance of the assay.** • After removing the 70% ethanol, the tube can be pulse-spun to allow removal of residual ethanol. • Take care not to disturb the DNA pellet; it may be small or invisible. • Should the pellet detach, centrifuge the sample for 5 minutes at 15,000 × *g*. • Excessive drying of the pellet can make the DNA more difficult to dissolve.
13. Add 100 µL of TE solution (see page 5) to dissolve the DNA pellet. Vortex for at least 5 seconds.	• If a higher concentration of DNA is desired, 50 µL of TE should be used.
14. To ensure complete rehydration of the DNA, incubate at room temperature overnight followed by vortexing or at 50°C for 1 hour with occasional vortexing.	• Large amounts of high molecular weight DNA can be slow to rehydrate (dissolve) completely. • Incomplete rehydration of the DNA is a cause of inaccuracy in estimating DNA concentration and potential failure of downstream applications such as PCR.
15. Options for storage of the fully rehydrated DNA: a) In TE at -20°C for long-term storage. Split into aliquots if desired. b) In TE at 4°C for up to 2 months.	

prepIT laboratory protocol
for manual purification of DNA from whole sample

Note: This protocol requires the use of a centrifuge (either fixed-angle or swinging-bucket rotor) capable of generating at least 3,500 × *g* to obtain optimal results.

The following step-by-step protocol describes how to purify DNA from the whole sample (1 mL-4 mL total sample volume). The volumes shown should be adjusted for the actual collected volume.

Reagents included
prepIT•L2P (Cat. No. PT-L2P-5 or PT-L2P-45)

Equipment and reagents
- Centrifuge that accommodates 15 mL tubes and is capable of generating at least 3,500 × *g* (see Table 2)
- 15 mL conical polypropylene tubes (e.g., BD Falcon® Cat. No. 352196)
- Microcentrifuge capable of running at 15,000 × *g* (optional)
- 1.5 mL microtubes (e.g., Axygen® Cat. No. MCT-150-C)
- Air or water incubator at 50°C
- Ethanol (95% to 100%) at room temperature
- Ethanol (70%) at room temperature
- DNA storage buffer: TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) or similar solution

Optional: Pre-purification check (only applicable for Oragene samples; not required for ORAcollect samples)
Weigh the sample to estimate the amount of saliva provided by the donor (see Table 1). The amount of saliva collected is directly proportional to the amount of DNA recovered. As an example, if a donor has provided less than 2 mL of saliva, you should expect to recover a lower total yield from this sample.

Weight of kit (without sample)
Once a sample arrives at the lab, we suggest weighing the sample to estimate if the right amount of saliva was provided by the donor. You can expect some variability across donors. The average weight of an empty kit is provided (Table 1). To estimate the amount of sample collected (assuming 1 g/mL), perform the following calculation:

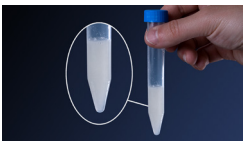
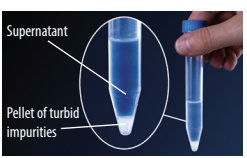
$$\frac{\textit{Weight of kit containing sample - Weight of kit without sample}}{\textit{Amount of sample collected}}$$

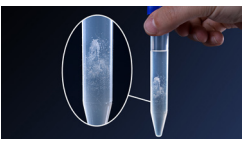
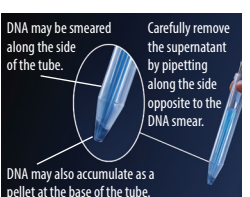
Table 1

Product #	Weight of kit without sample
OG-500/OGD-500/OGR-500	6.81 g
OG-510/OGD-510	5.83 g
OG-575/OGD-575/OGR-575	5.66 g
ON-500	6.47 g
ON-600	6.86 g
OG-600/OGD-600/OGR-600	7.26 g
OG-610/OGD-610	6.28 g
OG-675/OGD-675/OGR-675	6.00 g

Procedure

Purification steps	Notes
1. Mix the Oragene/ORAcollect sample by inversion or 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.	• This heat-treatment step is essential to maximize DNA yield and ensure that nucleases are permanently inactivated. • The sample may be incubated at 50°C overnight if more convenient. • This incubation step may be performed at any time after sample is collected and before DNA is purified. • A longer time is required in an air incubator because temperature equilibration is slower than in a water incubator. **Note:** The use of an air incubator may be preferable since the Oragene/ORAcollect 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.
3. Transfer the entire sample to a 15 mL centrifuge tube (Figure 1). Note the volume of the sample.  ***Figure 1**: Before proceeding to step 4, ensure that the entire sample has been incubated and transferred to a fresh 15 mL centrifuge tube, as shown.*	• Transfer can be carried out either by pouring or by pipetting with a glass or plastic pipette.

Purification steps	Notes
4. Add 1/25th volume of prepIT•L2P and mix by vortexing for a few seconds (Figure 2).  ***Figure 2**: After adding the PT-L2P and incubating on ice for 10 minutes, the sample will no longer look clear, but rather will be a cloudy solution.*	• E.g., to a 4 mL sample, add 160 µL of prepIT•L2P. • The sample will become turbid as impurities and inhibitors are precipitated.
5. Incubate on ice for 10 minutes.	• Room temperature incubation can be substituted but will be less effective at removing impurities.
6. Centrifuge at room temperature for 10 minutes at as high a speed as is possible. Minimum 3,500 × *g*.  ***Figure 3**: After centrifugation, there will be an accumulation of turbid material at the base of the tube. The supernatant should be visibly clear.*	• Higher centrifugal force minimizes the amount of turbid material that will be carried over into the purified DNA (Figure 3). Before proceeding, you should verify with the tube manufacturer that the 15 mL centrifuge tubes can withstand the centrifugal force. • A longer period of centrifugation (up to 20 minutes) may be beneficial in reducing the turbidity (high A_{320}) of the final DNA solution.
7. Carefully transfer the clear supernatant with a pipette to a fresh 15 mL centrifuge tube. **Discard the pellet.**	• Leave a small volume of the supernatant behind to avoid disturbing the pellet. • The pellet contains turbid impurities. If accidentally disturbed, the tube should be re-centrifuged.

Purification steps	Notes
8. Add 1.2x volume of room temperature 95% to 100% ethanol to the clear supernatant. Mix gently by inversion 10 times.  ***Figure 4**: After the addition of ethanol, the DNA will precipitate, which **may** result in a visible clot of fibres.*	• During mixing with ethanol, the DNA will be precipitated. • Precipitated DNA may appear as a clot of DNA fibres (Figure 4) or as a fine precipitate, depending on the amount of DNA in the sample.
9. Let the sample stand at room temperature for 10 minutes to allow the DNA to fully precipitate.	• Incubation at -20°C is not recommended because impurities may co-precipitate with the DNA.
10. Centrifuge at room temperature for 10 minutes at as high a speed as is possible. Minimum 3,500 × *g*.	
11. Carefully remove the supernatant with a glass or plastic pipette and discard it. Take care to avoid disturbing the DNA pellet.  ***Figure 5**: Using a pipette tip to gently scratch along the inside of the tube may reveal the presence of a DNA smear.*	• The supernatant may contain impurities and should be removed as completely as possible. • Precipitated DNA will be found as a pellet at the bottom of the tube and possibly as a smear down the side of the tube (Figure 5). • The DNA smear may be located on the side of the tube facing away from the centre of the centrifuge. • A smear can be located using the "scratch" test. You can check for the presence of a DNA smear by scratching the inside of the tube using a pipette tip. A smear, as shown in Figure 5, may be visible.

Purification steps	Notes
12. Ethanol wash: Carefully add 1 mL of 70% ethanol to the tube without disturbing the smear or the pellet. Let it stand at room temperature for 1 minute. **Gently swirl and completely remove the ethanol without disturbing the pellet and the smear.**	• **It is important to remove all ethanol from the sample. Carry-over of ethanol may impact the performance of the assay.** • Take care not to disturb the DNA pellet or the smear. • A short centrifugation (less than 1 minute) can be performed to facilitate complete removal of the supernatant. • Should the pellet detach after the ethanol wash step, centrifuge the sample for 5 minutes at as high a speed as is possible. Minimum 3,500 × *g*.
13. For Oragene samples, rehydrate the DNA by adding 0.2-1 mL of TE solution and vortex the sample for 30 seconds. For ORAcollect samples, rehydrate the DNA by adding 0.2 mL of TE solution and vortex the sample for 30 seconds.  ***Figure 6:*** *Vortexing the sample for 30 seconds will allow you to recover DNA smeared on the side of the tube. The DNA will remain high-molecular weight.*	• If a higher concentration of DNA is desired, the volume of TE may be reduced. A minimum of 200 µL TE solution should be used. • Excessive drying of the pellet (> 10 minutes) and using less than 500 µL of TE solution can make it difficult to rehydrate (dissolve) the DNA and may decrease the yield or make quantification difficult. • Precipitated DNA will be found as a pellet at the bottom of the tube and possibly as a smear down the side of the tube. • To ensure maximum DNA recovery, the sample must be vortexed after the addition of DNA solvent (TE solution). Vortexing will ensure that the DNA smeared on the side of the tube is recovered (Figure 6). • Vortexing will not shear the DNA.
14. To ensure complete rehydration of the DNA, incubate at room temperature overnight followed by vortexing or at 50°C for 1 hour with occasional vortexing.	• Incomplete rehydration of the DNA is a cause of inaccuracy in estimating DNA concentration and potential failure of downstream applications such as PCR.

Purification steps	Notes
15. Transfer the rehydrated DNA to a 1.5 mL microcentrifuge tube for storage.	
Optional step: a) Centrifuge the rehydrated DNA at room temperature for 15 minutes at 15,000 × *g*. b) Transfer the supernatant to a fresh 1.5 mL microcentrifuge tube without disturbing the pellet.	Note that the pellet contains insoluble, turbid material. • To maximize DNA recovery, ensure that the DNA is completely rehydrated (step 14) prior to performing this centrifugation step. • This centrifugation step ensures that any remaining turbid material is removed from the DNA sample. • Care should be taken not to disturb the pellet when transferring the clear supernatant to a fresh tube.
16. Options for storage of the fully rehydrated DNA: a) In TE at -20°C for long-term storage. Split into aliquots if desired. b) In TE at 4°C for up to 2 months.	• Freezing of purified DNA in TE may cause the DNA to precipitate. When thawing frozen purified DNA, pay careful attention to rehydration, as discussed in step 14.

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 suggest the use of commercially available kits such as the Quant-iT™ PicoGreen™ dsDNA Assay Kit (Thermo Fisher Scientific) or QuantiFluor® dsDNA System (Promega). DNA may need to be diluted up to 1:50 with TE before being 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*.[1] 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, double-stranded DNA.

Ensure that absorbance values are within the linear range of the spectrophotometer. Dilute and remeasure samples that fall outside of the linear range. See your instrument documentation for more information.

References

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

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} × 10 (dilution factor) × 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] × 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:
   $(A_{260} - A_{320}) \div (A_{280} - A_{320})$
   $= (0.295 - 0.025) \div (0.175 - 0.025)$
   $= 0.270 \div 0.150$
   = 1.80

Oragene•DNA and ORAcollect•DNA are not available for sale in the United States.
Oragene•DISCOVER is for research use only, not for use in diagnostic procedures.
Some DNA Genotek products may not be available in all geographic regions.

All DNA Genotek protocols, white papers and application notes are available in the support section of our website at www.dnagenotek.com.

Label legend:

IVD	In vitro diagnostic medical device
REF	Catalog number
CE	CE marking
	Manufacturer
	Consult package insert
EC REP	European Authorized Representative
CH REP	Swiss Authorized Representative
LOT	Lot number
UDI	Unique Device Identifier
	In-use stability
15°C – 30°C 59°F – 86°F	Storage instructions

Patent (www.dnagenotek.com/legalnotices)

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