Mini•SAL Salivary DNA Isolation Kit (Spin Column Method)

Catalog No. DNAX-504-30

Version 2.1, 20150710

Oasis Diagnostics® Corporation - *Pioneering Oral Fluid Diagnostic Solutions*

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Storage and Stability
All solutions should be kept tightly sealed and stored at ambient [room] temperature. If stored appropriately reagents should remain stable for at least 1 year in their unopened containers.

Material Safety and Data Sheets
Material Safety and Data Sheets (MSDS) for kit chemical components are available from Oasis Diagnostics® Corporation. Call 360-546-1563. Some Reagents included in this kit are irritants. The digestion buffer contains guanidine salts and is not compatible with disinfecting reagents containing bleach, strong acids or oxidizers.

Caution: Do not add bleach or acidic solutions directly to the sample-preparation waste. The sample-preparation waste can form highly reactive compounds when combined with bleach

Legal Notices
 The PCR process is covered by patents owned by Hoffman-La Roche Inc., and F. Hoffman-La Roche Ltd.

Warranty
Oasis Diagnostics® Corporation warrants that the products described in this manual meet the performance standards described in literature published by the company. If a product fails to meet these performance standards, Oasis Diagnostics® Corporation will replace the product or issue credit for the full purchase price, including delivery charges. Oasis Diagnostics® Corporation provides no other warranties of any kind, expressed or implied. Oasis Diagnostics® Corporation warranty liability shall not exceed the purchase price of the product and shall not extend to direct, indirect, consequential or incidental damages arising from the use, results of use, or improper use of its products.

Intended Use
This Kit is for Research Use Only. Not for use in diagnostic procedures.

DNA ISOLATION PROCEDURE FROM THE DNA·SAL™ SALIVARY DNA COLLECTION DEVICE

Overview

The Mini•SAL™ Saliva DNA Isolation Kit from Oasis Diagnostics® Corporation has been optimized to provide high-quality genomic DNA from saliva and/or buccal cells collected using the DNA•SAL™ Salivary DNA Collection Device [Catalog Number DNAS-102]. The Mini•SAL™ has been optimized for 0.1-1 mL sample volumes. The kit may be used effectively with other methods of saliva collection, subject to satisfactory validation. *NOTE: The sample collected using the DNA•SAL™ Salivary DNA Collection Device [Catalog Number DNAS-102], is considered a mixture of saliva, epithelial cells and stabilization solution.*

For processing larger sample volumes, 1–3mL, the Midi•SAL™ Saliva DNA Isolation Kit [Catalog Number DNAX-505] is recommended. The Midi•SAL™ has been optimized for 1-3mL sample volumes and can accommodate the full DNA•SAL™ collection volume in a single isolation. The DNA isolated using either the Mini•SAL or Midi•SAL™ Saliva DNA Isolation Kits is ideal for PCR, hybridization, sequencing, and other downstream applications.

In the Oasis Diagnostics® Mini•SAL™ purification procedure, DNA is isolated and purified using the **Mini•SAL™ Spin Column Assembly** (Figure 1) which contains an attachable high volume reservoir for sample loading. The Mini•SAL™ purification procedure comprises 4 main steps (Figure 2), Digest, Bind, Wash & Elute. The procedure can be carried out using a standard centrifuge, microcentrifuge, or vacuum manifold.

Figure 1: Mini•SAL™ Spin Column Assembly.

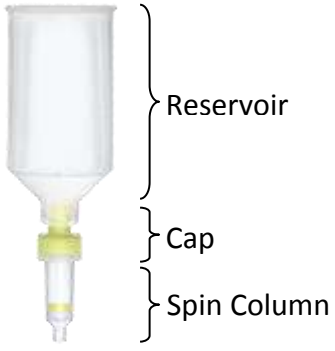
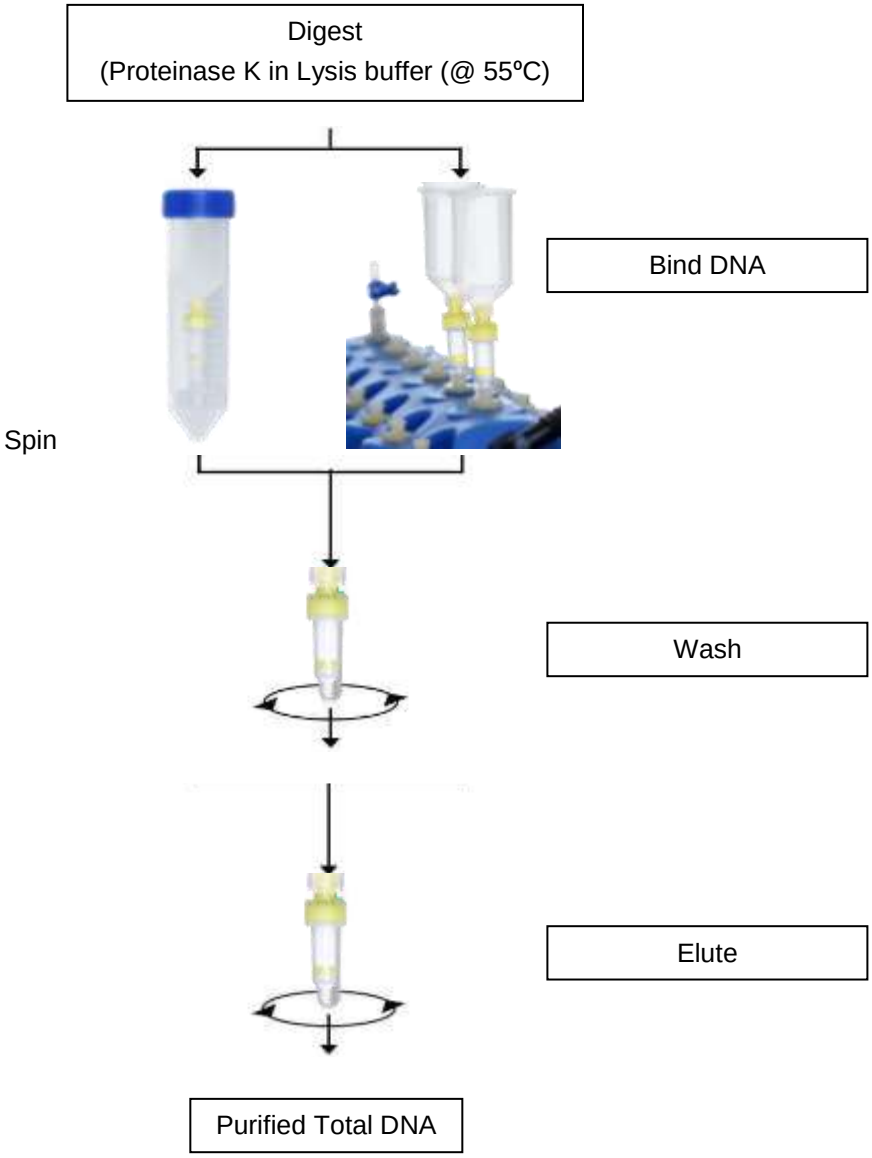


Figure 2: Oasis Mini•SAL™ Purification Steps.



Precautions

Please take the following precautions:

- Follow Good Laboratory Practices [GLP] when using the Oasis Diagnostics® Mini•SAL™ Salivary DNA Isolation Kit.

- Wear suitable Personal Protection Equipment, lab coat, disposable gloves and protective goggles. Follow Safety guidelines and rules enacted by your institution or facility.

- ***Caution: Do not add bleach or acidic solutions directly to the sample-preparation waste. The sample-preparation waste can form highly reactive compounds when combined with bleach.*** For more information, please consult the appropriate Material Safety Data Sheets (MSDS).

Handling DNA

Please take the following precautions to avoid DNA degradation:

- Employ proper microbiological aseptic techniques when working with DNA.
- Wear disposable gloves to prevent nuclease contamination from the surface of the skin.
- Use sterile, disposable plastic ware and automatic, aerosol-resistant pipettes reserved for DNA work. Wipe pipettes with DNase-removal solutions when transitioning between handling crude extracts to handling more purified material.

Saliva sample volumes ranging from 0.1 ml to 1 mL can be processed using the Standard Volume Protocol and the Mini•SAL™ Spin Column Assembly with reservoir. With the reservoir, standard volume samples can be loaded in a single column addition using a centrifuge or vacuum manifold. In the event a centrifuge or vacuum manifold is not available, the reservoir can be detached and the sample can be loaded by multiple additions to the column using a microcentrifuge (< 850 µL per addition), see Table 3. Column wash and elution steps can be performed using a microcentrifuge.

Kit Contents

Items Provided

Table 1: Kit Contents

Component	Quantity	Storage
Proteinase K	3 X 20 mg	-20°C (after mixing)
Proteinase K Storage Buffer	3 X 1.2 mL	Room Temp
Digestion Buffer	1 X 30 mL	Room Temp
Binding Buffer	1 X 60 mL	Room Temp
DNA Wash Buffer 1	1 X 15 mL	Room Temp
DNA Wash Buffer 2	1 X 50 mL	Room Temp
Elution Buffer	1 X 4 mL	Room Temp
Column & Reservoir Assemblies	30	Room Temp
Collection Tubes	30	Room Temp
Instructions	1	Room Temp

Note: Kit contains sufficient material for isolating DNA from thirty (1.0) mL samples.

Items Required But Not Provided

Microcentrifuge (10,000 x g with rotor for 2 mL tubes), centrifuge, and/or vacuum manifold.
Microcentrifuge tubes (1.5 mL or 2 mL)
50 mL disposable conical centrifuge tubes
Heat block, incubator or water bath (55°C)

Buffer Preparation

Proteinase K Solution Preparation

Add 1 mL (1,000 µL) **Proteinase K Storage Buffer** to each **Proteinase K** tube prior to use. The final concentration of **Proteinase K** after the addition of **Proteinase K Storage Buffer** is ~20 mg/mL.

Protocol

Saliva sample volumes ranging from 0.2 mL to 1.0 mL can be processed using the Standard Volume Protocol and the Mini•SAL™ Spin Column Assembly with reservoir. With the reservoir, standard volume samples can be loaded in a single column addition using a centrifuge or vacuum manifold. In the event a centrifuge or vacuum manifold is not available, the reservoir can be detached and the sample can be loaded by multiple additions to the column using a microcentrifuge (< 850 µL per addition), see Table 3. Column wash and elution steps can be performed using a microcentrifuge. Samples may also be pelleted and processed using the Pellet Protocol

Table 2: Recommended Protocols.

Sample Volume Input	Protocol	Equipment Needed
0.2 – 1 mL	Standard Volume	Centrifuge and Microcentrifuge* or Vacuum Manifold (e.g. Vac-Man™ Promega)
	Pellet Protocol	Microcentrifuge only
≤ 0.2 mL	Small Volume	Microcentrifuge only

Centrifuge must accommodate 50 mL conical tubes
Vacuum Manifold operating range: ≥ 350 mm Hg

**The sample can be loaded onto the column multiple times in the event a centrifuge or vacuum manifold is not available. Reservoir must be detached when using a microcentrifuge.*

Standard Volume Protocol (0.2 –1.0 mL)

1. To 500 µL of collected saliva sample in a conical tube, add :

Digestion Buffer	450 µL
Proteinase K	50 µL

Note: The digestion buffer and proteinase K must be scaled to accommodate other sample volumes. The digestion buffer containing Proteinase K is added at an equal volume to the sample volume. Proteinase K is added at a volume 0.1X the sample volume, see Table 3. (e.g., Add 0.9 mL Digestion Buffer and 100 µL of Proteinase K to 1 mL saliva sample).

2. Mix well and incubate the tube at 55° C for 20 minutes.

3. Add 1 volume of **Binding Buffer** to the digested sample: *sample volume + digestion buffer volume (*e.g., 1 mL binding buffer to 1mL digested sample). Mix thoroughly.

4. Load mixture onto the column:

Caution: Make sure the connection between the column and reservoir assembly is secure, finger tight.

   a. Centrifugation: Place the **Spin-column-cap-reservoir** assembly into a 50 mL conical centrifuge tube. Transfer the mixture to a **Spin Column-Cap-Reservoir** assembly. Spin at 250 x g for 5 minutes in a swinging bucket rotor until solution has passed through the column. Discard flow-through.

   b. Vacuum manifold (sample input of ≤ 0.5 ml, mixture ≤ ~2 mL): Place **Spin-column-Cap-reservoir** on vacuum manifold. Transfer the mixture to a **Spin Column-Cap-Reservoir** assembly. Apply vacuum, ≥ 380 mm Hg until the entire solution has passed through the column. Discard flow-through

Note: Alternatively, the digestion mixture can be processed without using the reservoir but will require multiple additions of ≤ 850 *µL* per addition, see table 3.

5. Remove and discard the **Cap** and **Reservoir** from the **Spin Column.** Transfer the **Spin Column** to a **Collection Tube**.

6. Add 400 µL of **Wash Buffer 1** to the column. Centrifuge for one minute at 10,000 *x g* in a microcentrifuge. Discard flow-through.

7. Add 700 µL of **Wash Buffer 2** to the column. Centrifuge for one minute. Discard flow-through.

8. Add 400 µL of **Wash Buffer 2** to the column. Centrifuge for two minutes. Discard flow-through.

9. Transfer the column to a clean microcentrifuge tube. Add 100 µL **Elution Buffer** or water* directly to the column matrix. Incubate 5 minutes at room temperature and then centrifuge at top speed (10,000 *x g*) for 30 seconds to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤-20ºC for future use.

*__Note__: Elution of DNA from the column is dependent on pH and temperature. If water is used, ensure the pH is > 6.0. Total yield may be improved by eluting the DNA with elution buffer or water warmed to 60-70^{o}C.

Small Volume Protocol (0.1 mL – 0.2 mL) ***(microcentrifuge only)***

1. To a 200 µL saliva sample in a conical tube, add:

Digestion Buffer	180 µL
Proteinase K	20 µL

Note: Digestion can be scaled down for smaller volumes of sample (e.g., To 100 µL saliva sample add 90 µL Digestion Buffer and 10 µL of Proteinase K).

2. Mix well and incubate the tube at 55° C for 20 minutes.

3. Add 1 volume of **Binding Buffer** to the digested sample: *sample volume + digestion buffer volume (*e.g., 1 mL binding buffer to 1mL digested sample). Mix thoroughly.

4. Remove cap and reservoir. Place **Spin Column** in a **Collection tube**. Load mixture onto the column. Centrifuge at ≥ 10,000 *x g* for 1 minute in a microcentrifuge. Discard flow-through. *(If using more than 200 µl of sample, the column can be loaded column multiple times, see table 3)*

5. Transfer the **Spin Column** to a new **Collection Tube**.

6. Add 400 µL of **Wash Buffer 1** to the column. Centrifuge for one minute at 10,000 x *g* in a microcentrifuge. Discard flow-through.

7. Add 700 µL of **Wash Buffer 2** to the column and centrifuge for one minute. Discard flow-through.

8. Add 400 µL of **Wash Buffer 2** to the column and centrifuge for two minutes. Discard flow-through.

9. Transfer the column to a clean microcentrifuge tube. Add 100 µL **DNA Elution Buffer** or water* directly to the column matrix. Incubate 5 minutes at room temperature and then centrifuge at top speed (10,000 *x g*) for 30 seconds to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤-20ºC for future use.

   ***Note**: Elution of DNA from the column is dependent on pH and temperature. If water is used, ensure the pH is > 6.0. Total yield may be improved by eluting the DNA with elution buffer or water warmed to 60-70^{o}C.

Table 3: Scaling and loading for microcentrifuge only without reservoir. With the reservoir, samples volumes > 200 µL can be loaded with a single addition

Sample Volume (µL)	Digestion buffer (µL)	Proteinase K (µL)	Binding Buffer (µL)	Total Volume (µL)	Load Additions to Column (≤ 850 µL)
100	90	10	200	400	1X
200	180	20	400	800	1X
400	360	40	800	1600	2X
500	450	50	1000	2000	3X
1000	900	100	2000	4000	5X

Note: volume must be ≤ 850 µL per addition.

Pellet Protocol (0.2 –1.0 mL)

1. Pellet collected sample at ≥ 500 x *g* for 5 minutes in a microcentrifuge tube.

2. Carefully aspirate and discard the supernatant.

3. To the pellet, add :

Digestion Buffer	100 µL
Water	100 µL
Proteinase K	20 µL

4. Mix well and incubate the tube at 55° C for 20 minutes.

5. Add 1 volume of **Binding Buffer** to the digested sample: *sample volume + digestion buffer volume (*e.g., 1 mL binding buffer to 1mL digested sample). Mix thoroughly.

6. Remove cap and reservoir from the column assembly. Place **Spin Column** in a **Collection Tube**. Load mixture from Step 5 onto the column. Centrifuge at ≥ 10,000 *x g* for 1 minute in a microcentrifuge. Discard flow-through.

7. Transfer the **Spin Column** to a new **Collection Tube**.

8. Add 400 µL of **Wash Buffer 1** to the column. Centrifuge for one minute at 10,000 *x g* in a microcentrifuge. Discard flow-through.

9. Add 700 µL of **Wash Buffer 2** to the column. Centrifuge for one minute. Discard flow-through.

10. Add 400 µL of **Wash Buffer 2** to the column. Centrifuge for two minutes. Discard flow-through.

11. Transfer the column to a clean microcentrifuge tube. Add 100 µL **Elution Buffer** or water* directly to the column matrix. Incubate 5 minutes at room temperature and then centrifuge at top speed (10,000 *x g*) for 30 seconds to elute the DNA. The eluted DNA can be used immediately for molecular based applications or stored ≤-20ºC for future use.

    ***Note**: Elution of DNA from the column is dependent on pH and temperature. If water is used, ensure the pH is > 6.0. Total yield may be improved by eluting the DNA with elution buffer or water warmed to 60-70^{o}C.

Typical Performance:

To assess performance equivalent volumes (0.2 mL) of sample collected using the DNA•SAL™ Salivary DNA device were extracted in triplicate using the Mini•SAL™ Salivary DNA Isolation Kit.

DNA yield was quantified using a NanoDrop® spectrophotometer and by quantitative PCR using Syto9®. DNA quality was determined by A_{260}/A_{280} ratio, A_{260}/A_{230} ratio, agarose gel electrophoresis and by quantitative PCR performance (β-actin). DNA yield by PCR was quantified using a Human DNA standard curve.

Typical yield and performance results are displayed in Table 4 and Figure 3. The isolation yields *High Quality* DNA with an average size of ≈ 30 kb suitable for down stream applications such as qPCR. The Mini•SAL™ isolation kit provides greater flexibility in sample volume processing than other similar kits which have restricted volume capacity. With the Mini•SAL™ isolation kit sample volumes of 0.1 to 1 mL can be processed in a single setting.

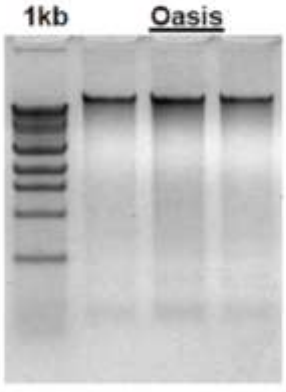
Table 4: Characterization of DNA isolated from 0.2 mL of saliva sample.

Data	Avg	STD dev
Yield (µg) NanoDrop® (A_{260})	2.21	0.38
Yield (µg) qPCR (β-actin)	2.10	0.26
A_{260}/A_{280} ratio	1.85	0.03
A_{260}/A_{230} ratio	2.38	0.24
qPCR* (β-actin) Ct	22.66	0.38

*Note: The PCR was performed using ZymoTaq Premix supplemented with Syto9 (final concentration = 2.5 µM) using a Lightcycler 480 (Roche). DNA was quantified from a standard curve generated using human DNA. Beta-actin primer pair: 353 bp amplicon.

1. Forward: GCTCTGTCGTCGACAACGGCTC
2. Reverse: CAAACATGATCTGGGTCATCTTCTC
3. Annealing temperature: 59°C.

Figure 3. Agarose gel electrophoresis of DNA isolated using the Mini•SAL™ isolation kit.



Size of DNA extracted ≈ 30 kb (fresh sample)

RELATED PRODUCTS

Related Products- Ordering Information	Catalog Number
Mini•SAL™ DNA Isolation Kit [30 Preparations]	DNAX-504-30
Midi•SAL™ DNA Isolation Kit [30 Preparations]	DNAX-505-30
DNA•SAL™ Salivary DNA Collection Tool	DNAS-102
RNAPro•SAL™ Salivary RNA and Protein Collection Device	RPSAL-701
Proteinase K	PROK-503

Technical Support

For Technical Support [available between 8.00 a.m. and 5.00 p.m. Pacific Standard Time] Monday through Friday, please call Oasis Diagnostics® Corporation at (360) 546-1563. Technical support may also be obtained by sending details of any requirements by e-mail to info@4saliva.com A Troubleshooting Guide for the Salivary DNA Extraction Kit [96-Well Microplate Method] is available upon request.

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