# Midi•SAL™ Salivary DNA Isolation Kit (Spin Column Method)

Catalog No. DNAX-505-30

Version 1.2

**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 SAMPLE KIT FOR 30 PREPARATIONS FROM 3.0 ML SAMPLES

## Overview

The Midi•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 Midi•SAL™ has been optimized for 1-3 mL sample volumes and can accommodate the full DNA•SAL™ collection volume in a single isolation. 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 smaller sample volumes, 0.1 – 1mL, the Mini•SAL™ Saliva DNA Isolation Kit [Catalog Number DNAX-504-30] is recommended. The Mini•SAL™ has been optimized for 0.1-1mL sample volumes. The DNA isolated using either the Midi•SAL or Mini•SAL™ Saliva DNA Isolation Kits is ideal for PCR, hybridization, sequencing, and other downstream applications.

In the Oasis Diagnostics® Midi•SAL™ purification procedure, DNA is isolated and purified using the **Midi•SAL™ Spin Column Assembly** (Figure 1) which contains an attachable high volume reservoir for sample loading. The Midi•SAL™ purification procedure comprises 4 main steps (Figure 2), Digest, Bind, Wash & Elute. The procedure is carried out using a large volume capacity centrifuge with a swinging bucket rotor and a microcentrifuge.

**Figure 1: Midi•SAL™ Spin Column Assembly.**

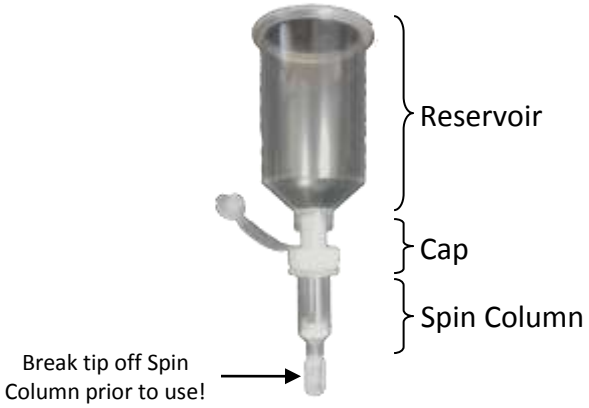
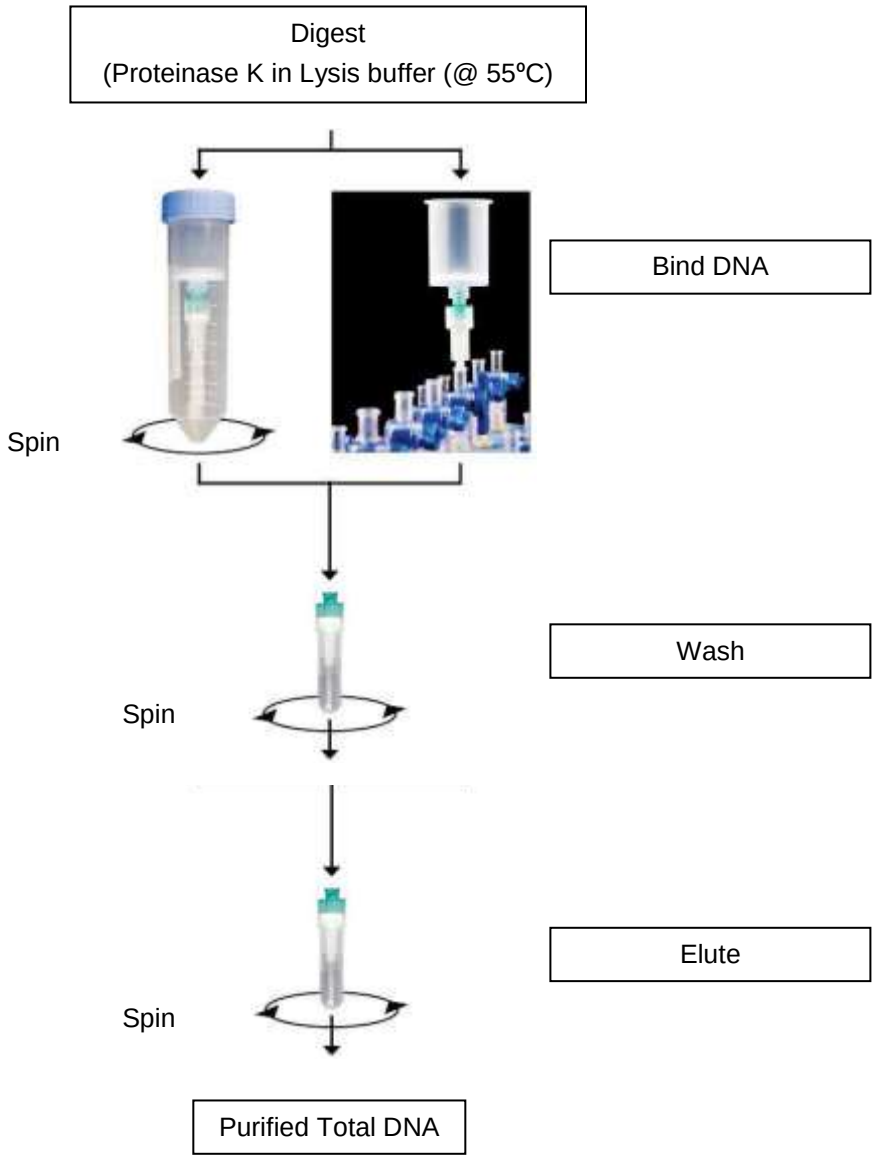


**Figure 2: Oasis Midi-Sal Purification Steps.**




## Precautions

Please take the following precautions:

- Follow Good Laboratory Practices [GLP] when using the Oasis Diagnostics® Midi•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.

## 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 1.5 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 50 mL | Room Temp |
| DNA Wash Buffer 2 | 1 X 60 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 30 [thirty] 3.0 mL samples.*

### Items Required *But Not Provided*

Centrifuge with swinging bucket rotor
Microcentrifuge (10,000 x g)
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**

**Standard Volume Protocol (*1.0 –3.0 mL)***

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

| | |
|---|---|
| Digestion Buffer | 0.9 mL |
| Proteinase K | 0.1 mL |

**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 of the sample volume (e.g., Add 2.7 mL Digestion Buffer and 0.3 mL Proteinase K to 3 mL saliva sample; total volume 6 mL).

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, *see sample scaling and loading table 2*). Mix thoroughly.

4. Place the **Spin-Column-Reservoir** assembly into a 50 mL conical centrifuge tube.

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

5. Transfer up to 15 mL of the mixture to a **Spin Column-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. Repeat until all of the mixture has passed through the column.

6. Add 5 mL of **Wash Buffer 1** to the **Spin Column-Reservoir** assembly. Spin at Spin at 250 x g for 2 minutes.

7. Add 5 mL of **Wash Buffer 2** to the **Spin Column-Reservoir** assembly. Spin at Spin at 250 x g for 2 minutes.

8. Remove Reservoir and transfer the column to a **Collection Tube**.

9. Add 0.2 mL of **Wash Buffer 2** to the Spin Column. Spin at top speed (>10,000 x *g*) for 1 minute in a microcentrifuge.

10. Transfer the **Spin Column** to a clean microcentrifuge tube.

11. Add 200 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 in a microcentrifuge 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°C. An additional elution may increase total recovery.

**Table 2: Scaling and loading for various sample volumes.**

| Sample Volume (mL) | Digestion buffer (mL) | Proteinase K (mL) | Binding Buffer (ml) | Total Volume (ml) |
|---|---|---|---|---|
| 1 | 0.9 | 0.1 | 2 | 4 |
| 2 | 1.8 | 0.2 | 4 | 8 |
| 3 | 2.7 | 0.3 | 6 | 12 |

## RELATED PRODUCTS

| Related Products- Ordering Information | Catalog Number |
|---|---|
| Mini•SAL™ Salivary DNA Isolation Kit [30 Preparations] | DNAX-504-30 |
| 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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