**User Guide**

# PureBind Swab DNA Isolation Kit

For isolation and purification of genomic and mitochondrial DNA from human buccal swab samples.

**Version** **190422**

**Catalog** **K1619-SMP**

**K1619-100**

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## Product description

The PureBind Swab DNA Isolation Kit provides a simple, highly reproducible, and automation-ready route to microgram quantities of high molecular weight genomic and mitochondrial DNA from buccal swab samples, with typical isolations yielding greater than 6 ug DNA from Puritan® HydraFlock® swabs. The system utilizes magnetic bead technology, enabling efficient, consistent, and scalable extraction of DNA. The extracted DNA is free from protein and other contaminants, making it suitable for a variety of downstream applications, such as PCR, Real-time PCR, NGS sequencing, enzymatic digestion, or molecular cloning.

## Contents and storage

Reagents have a one (1) year expiration date from date of purchase when stored properly.

| PureBind Blood DNA Isolation Kit<br>Catalog no. | 4 preps [3]<br>K1619-SMP | 100 preps [3]<br>K1619-100 | Storage |
|---|---|---|---|
| PureBind Beads [1] | 100 ul | 3 ml | 2-8°C |
| Digestion Buffer [1] | 2 ml | 40 ml | 15-30°C |
| Lysis/Binding Buffer [1] | 2 ml | 40 ml | 15-30°C |
| Wash Buffer 1 [1, 2]<br>*Customer adds Isopropanol* | 6 ml | 2 x 60 ml | 15-30°C |
| Wash Buffer 2 [1, 2]<br>*Customer adds Ethanol* | 3 ml | 2 x 30 ml | 15-30°C |
| Elution Buffer [1] | 500 ul | 10 ml | 15-30°C |

**Note**. Some reagents in each kit are provided in excess of the amount required.

[1] PureBind Beads are also available as Catalog no. A1602 and Lysis/Binding Buffer is available as Catalog no. R1247 and Wash Buffer 1 is available as Catalog no. R2340 and Wash Buffer 2 is available as R2355, and Elution Buffer is available as R0510.

[2] Prior to first time use, add the required amount of alcohol as indicated on the label of each bottle, then check the box and mix well.

[3] The number of preps or isolations are based on a 1 buccal swab sample.

## Required materials not supplied

| Item | Source | Catalog no. |
|---|---|---|
| Ethanol, absolute (100%) | Fisher Scientific | BP291914 |
| Isopropanol (100%) | Sigma Aldrich | I9516 |
| Proteinase K, lyophilized | Gold Biotech | P-480-100 |
| Micro- and conical tubes (1-15 ml) | Various | Not applicable |
| Magnetic separator for micro-tubes | Ocean NanoTech | MMS |
| Vortex mixer | Various | Not applicable |
| Heat block for tubes | Various | Not applicable |
| Rotator (End-Over-End Mixer) | Thermo Fisher | HulaMixer |
| Pipettors (20-1000 ul) and tips | Various | Not applicable |
| Buccal cell collection swabs | Puritan | 25-3406-H |

## Principle

The magnetic bead technology and solutions in this kit are used in a simple separation protocol to isolate genomic and mitochondrial DNA from buccal swab samples: a chemo-enzymatic lysis of captured cells is followed by incubation with beads in the presence of isopropanol. Beads bind to DNA and are thereafter easily separated from the lysate using a magnet. Magnetic separation facilitates simple washing and elution of the isolated DNA.

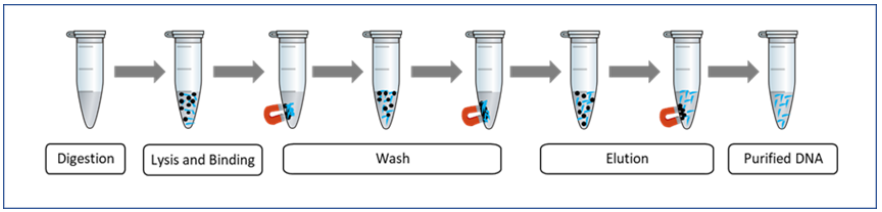


**Figure**. Illustration showing magnetic separation of DNA from a human buccal cell swab sample using the PureBind Swab DNA Isolation Kit.

## Procedural guidelines

- Read the USER GUIDE and make sure all the directions are followed and all recommended volumes are used as indicated.
- Prior to use, bring all kit components to room temperature.
- All vortex steps should be performed at maximum speed to ensure mixing.
- Do not add proteinase K directly to the Lysis/Binding Buffer.
- PureBind Beads should be resuspended to a homogenous suspension prior to use.

## Before you begin

**Prepare working buffers**

- Prior to first time use, add isopropanol (100%) as indicated on the label of each bottle(s) of Wash Buffer 1 to obtain a working solution. Check the box on the label and mix well by inverting 10 times.
- Prior to first time use, add ethanol (100%) as indicated on the label of each bottle(s) of Wash Buffer 2 to obtain a working solution. Check the box on the label and mix well by inverting 10 times.

**Prepare proteinase K solution**

Prepare enough proteinase K solution at 20 mg/ml (not provided) by dissolving lyophilized proteinase K powder in 50 mM Tris (pH 8.0). Then divide the stock solution into small aliquots and store at -20°C. Alternatively, a 20 mg/ml proteinase K solution can be purchased from Gold Bio (Catalog no. P-480-SL2) or a different supplier.

**Collection of Buccal Swab**

Restrict donor from eating food or drink beverages (excluding water) for at least 30 minutes prior to collection time. Also, put on sterile gloves and wear them through the collection procedure.

1. Rinse mouth with deionized water for 15 seconds.
2. Remove a swab from its sterile packing tube. Do not allow the swab to touch any object or surface.
3. With the swab in between the forefinger and thumb, insert the brush into one side of the mouth between the inside of the cheek and the upper gum. Press firmly and twirl the swab head against the inside of

the inner cheek using an up and down motion, moving the swab head from front to back and back to front. Do this for at least 30 seconds per swab to collect buccal cells.
*Caution. Avoid saturating the swabs with excess saliva.*

4. Remove the swab from the donor's mouth and place it in a sterile, DNase-free microcentrifuge tube. Break off the swab head at the pre-scored break site and allow the swab to air dry on for a least five minutes.
   Note. Swab samples are ready for immediate use. Alternatively, you can store the swab sample in a micro tube at 4°C for next-day use or -20°C for long-term storage.

## Protocol: Isolate DNA from buccal swab samples

The following protocol is for DNA isolation from a buccal swab sample. The protocol has been tested extensively with Puritan® HydraFlock® swabs but other dry flock swab types may work with the following protocol. The user may need to add more Digestion Buffer and/or Lysis/Binding Buffer depending on the intrinsic volume retention of the swab being evaluated.

**Lyse sample and bind PureBind Beads to genomic DNA**

1. Add 1 dried swab head to a sterile micro tube.
2. Add 350 ul Digestion Buffer and 10 ul Proteinase K (20 mg/ml). Vortex for 10 seconds to mix. Then incubate for 2 minutes at room temperature.
3. Add 350 ul Lysis/Binding Buffer. Vortex for 10 seconds to mix. Then incubate for 20 minutes at 56°C followed by a final vortex for 10 seconds.
4. Add 25 ul PureBind Beads and 400 ul Isopropanol. Vortex for 10 seconds to mix. Then place the mixture in a rotator (end-over-end) for 3 minutes to bind DNA to the beads, followed by a final vortex for 10 seconds.
5. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.

**Wash PureBind Beads with wash buffers**

6. Remove the tube from magnetic separator. Add 1 mL of Wash Buffer 1 and vortex for 10 seconds.
7. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.
8. Repeat Steps 6-7 for a second wash with the Wash Buffer 1.
9. Remove the tube from magnetic separator, add 1 mL of Wash Buffer 2 and vortex for 10 seconds.
10. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.
11. Repeat Steps 9-10 for a second wash with Wash Buffer 2.
12. Keep the tube on the magnetic separator with the lid open. Air dry the beads for 5 minutes. Remove any visible supernatant without disturbing the beads.

    *Caution. Do not over dry beads as it may result in loss of cfDNA.*

**Elute the cfDNA**

13. Remove the tube from magnetic separator and add 100 ul of Elution Buffer. Completely resuspend beads by pipetting 10 times. Then incubate for 2 minutes at room temperature.

    *Note. To increase DNA yields, incubate at 56°C for 3 minutes.*
14. Place the tube on the magnetic separator for 2 minutes or until the solution clears and beads are pelleted.
15. Carefully transfer the supernatant containing the purified genomic DNA without disturbing the pellet to a clean, labeled microcentrifuge tube.

    *Note. The purified DNA is ready for immediate use. Alternatively, you can store the DNA at 4°C for up to 24 hours or -20°C for long-term storage.*

## Yield and quality measurements

### DNA yield

We recommend using the Invitrogen™ Qubit™ dsDNA BR Assay Kit (Catalog no. Q32850 for total genomic DNA yield and quantification. This assay is specifically designed for use with the Qubit Fluorometer but can be used with any fluorometer or fluorescence plate reader.

### DNA quality

We recommend using the NanoDrop or any spectrophotometer to determine the DNA quality. The NanoDrop is able to measure a sample using just 1 ul of sample and in less than five seconds to assess the purity of the sample such as proteins and nucleic acids.

## Document History

| Version | Person | Description of Change |
|---|---|---|
| 150725 | Luis Moreno | Initial draft |
| 180718 | Luis Moreno | Consultation draft – to Alice Bu |
| 190422 | Luis Moreno | Final version – approved by Luis Moreno |

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## Warranties and Disclaimers

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