

## Swab DNA Preparation - Column Kit

### DNA purification from swabs with silica-membrane columns

#### Genomic DNA Preparation

Cat. No.	Amount
PP-238S	50 preparations
PP-238L	250 preparations

#### For general laboratory use

**Shipping:** shipped at ambient temperature

**Storage Conditions:** store at ambient temperature

**Shelf Life:** 12 months

#### Content

Component	PP-238S 50 preps	PP-238L 250 preps
Lysis Buffer	16 ml	80 ml
RNase A <u>store RNase A solution at -20°C</u>	7.5 mg <b>add 150 µl ultra-pure water</b>	5 x 7.5 mg <b>add 150 µl ultra-pure water to each tube</b>
Proteinase K <u>store Proteinase K solution at -20°C</u>	5 mg <b>add 500 µl ultra-pure water</b>	5 x 5 mg <b>add 500 µl ultra-pure water to each tube</b>
Binding Buffer	16 ml	80 ml
Activation Buffer	6 ml	30 ml
Washing Buffer	<b>add 48 ml Ethanol</b> (final volume 60 ml)	<b>add 120 ml Ethanol to each bottle</b> (final volume 150 ml)
Elution Buffer	5 ml	25 ml
Spin Columns	50	250
Collection Tubes	50	250

#### To be provided by you

- 96-99 % Ethanol
- ultra-pure water
- microtubes 1.5 or 2.0 ml
- heating block or water bath at 60 °C

**Before start, add the following components** (not included in the kit) as indicated in the **Content** table and/or on the bottle/tube:

- ultra-pure water to RNase A and Proteinase K (store soluted enzymes at -20°C)
- 96-99 % Ethanol to the Washing Buffer

#### Description

Swab DNA Preparation - Column Kit is designed for rapid and high purity isolation of genomic DNA from mucosal swabs i.g. buccal, nasal or throat swabs.

The kit also allows the extraction of DNA from swabs taken from surfaces of objects such as door handles, taps, handholds, etc.

Before taking samples from dry surfaces, moisten the sample stick with PBS or purified water. Wipe the swab over the surface to be tested.

Column-based purification completely removes PCR inhibitors such as divalent cations and proteins resulting in a high purity preparation of genomic DNA. There is no use of phenol or chloroform, handling is safe and does not produce harmful waste.

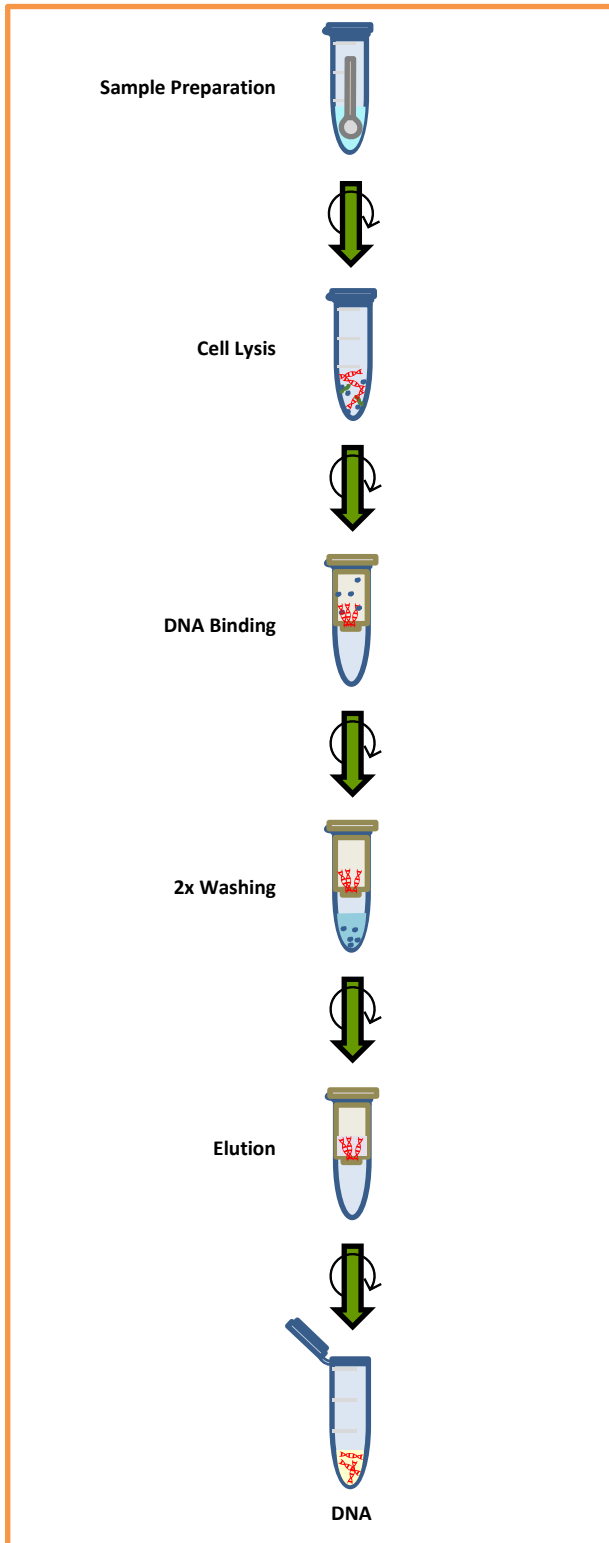
#### Application

The Swab DNA Preparation Kit allows extraction of usually up to 2 µg of highly pure genomic DNA in a size range from 200 bp to 50 kb per preparation. The obtained DNA is suitable for a variety of applications, including real-time PCR, southern blot analysis, genotyping and discovery or validation of SNP/SSR markers.

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### DNA Preparation

#### 1) Sample Preparation and

- Add **300 µl Lysis Buffer** and **2 µl RNase A** to a micro-centrifuge tube
- Take the cotton swab and cut off the tip with the collected mucosal cells / cells from a surface
- Place the cotton tip in the micro-centrifuge tube

#### 2) Cell Lysis

- Vortex for 30 sec
- Incubate at 60 °C for 5 min
- Add **8 µl Proteinase K** and mix by pipetting
- Incubate at 60 °C for 10 min
- Cool down to room temperature
- Remove the cotton tip and squeeze it out at the rim of the tube

#### 3) DNA Binding

- Add 300 µl Binding Buffer and mix by inverting
- Place the tube on ice to cool down
- Centrifuge for 5 min at 10,000 g
- Transfer the supernatant into a new tube
- Add **500 µl Ethanol (not included in the kit)**
- Mix by pipetting up and down

#### 4) Column Activation

- Place a spin column into a 2 ml Collection Tube
- Add **100 µl Activation Buffer** into the Spin Column
- Centrifuge at 10,000 g for 30 sec
- Discard flow-through

#### 5) Column Loading

- Pipet 600 µl supernatant from **DNA Binding** directly into the Spin Column (to avoid overloading of the column)
- Centrifuge for 1 min at 10,000 g
- Discard the flow-through
- Repeat the step with remaining supernatant

#### 6) Washing

- Add **500 µl Washing Buffer** into Spin Column
- Centrifuge for 30 sec at 10,000 g
- Discard the flow-through

**Optional: Repeat Washing if highest purity is required**

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##### 7) Remove residual Washing Buffer

- Centrifuge at 10,000 g for 2 min to remove residual Washing Buffer
- Discard the 2 ml Collection Tube
- Place the Spin Column into a new 1.5 ml microtube

##### 8) Elution of DNA

- Add **50 µl Elution Buffer** into the center of the Spin Column
- Incubate at room temperature for 1 min
- Centrifuge at 10,000 g for 1 min
- DNA in Elution Buffer is stable at 4 °C or -20 °C for several months

#### Troubleshooting

Problem	Cause	Suggestions
Low yield	Insufficient lysis	Reduce the amount of starting material. Prolong the lysis step. Shake the sample during incubation.
	No Ethanol added to Washing Buffer	Make sure to add Ethanol to the Washing Buffer before start.
	DNA is sheared / degraded	Avoid excessive pipetting after lysis. Do not vortex after lysis.
	DNA is not completely eluted	Elute in two steps. Increase the volume of Elution Buffer. Prolong the incubation of the Elution Buffer on the Spin Column to 5 min. Make sure the Elution Buffer is delivered onto the matrix and not on the wall of the Spin Column.
RNA contamination	No / not enough RNase added	Make sure to add RNase A in the Cell Lysis step.
Inhibition of downstream enzymatic reactions	Ethanol carryover	Make sure to remove residual Washing Buffer before elution. Remove the Spin Column carefully from the Collection Tube so that it does not come in contact with the flow-through. If in doubt, centrifuge again for 1 min.