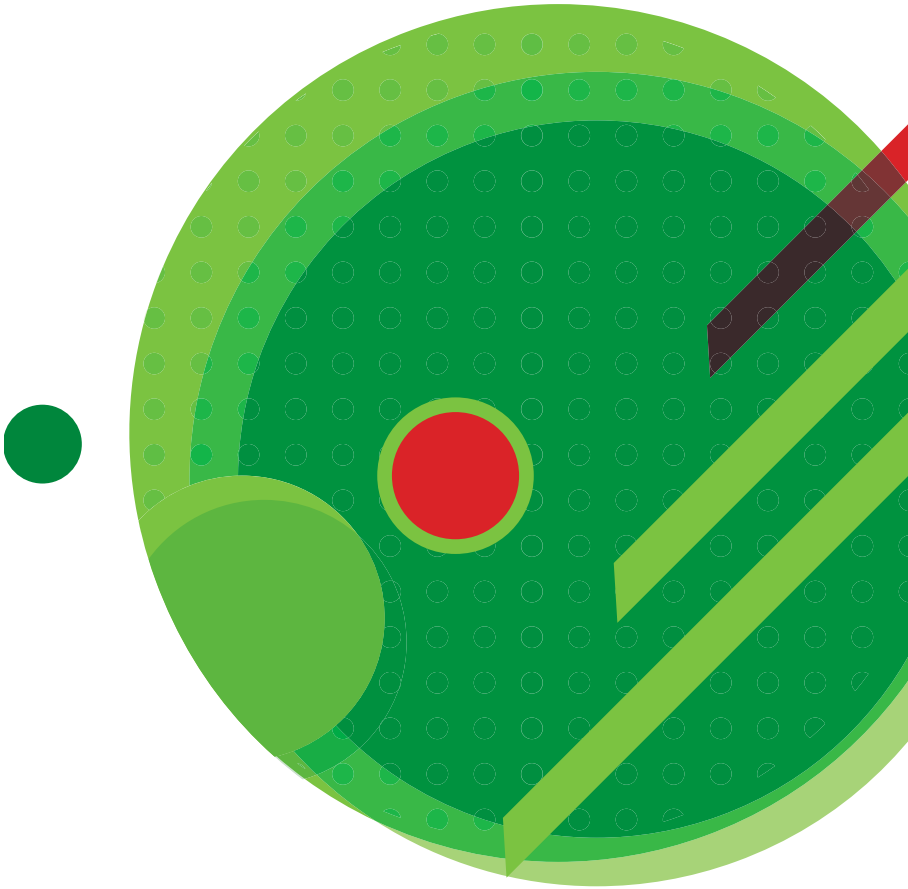
EXTRACTME®
DNA SWAB & SEMEN KIT

Cat. No. EM06
Version: 1.2018

Kit for genomic DNA isolation from swabs and semen



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I. INTENDED USE

The ***EXTRACTME*** **DNA SWAB & SEMEN KIT** is designed for a rapid and efficient purification of high quality DNA from human and animal mucosa membrane swabs (including buccal, nasal, pharyngeal and vaginal swabs) as well as from semen. The isolation protocol and buffer formulations were optimized for high isolation efficiency and DNA purity. The product is intended for research use only.

II. COMPONENTS OF THE KIT AND STORAGE CONDITIONS

NUMBER OF ISOLATIONS	10 ISOLATIONS	50 ISOLATIONS	250 ISOLATIONS	Storage Conditions[1]
Catalogue number	EM06-010	EM06-050	EM06-250	
SSL Buffer (Lysis Buffer)	3.5 ml	18 ml	88 ml	RT
⚠ **Proteinase K**[*] (lyophilized)	1 pc	1 pc	5 pcs	-20°C[2]
Proteinase Buffer	200 µl	320 µl	1.6 ml	RT
⚠ **DTT**[**]	1 pc	1 pc	1 pc	-20°C[3]
SSB Buffer (Binding Buffer)	3.5 ml	18 ml	88 ml	RT
SSW1 Buffer (Wash Buffer 1)	6 ml	30 ml	150 ml	RT
SSW2 Buffer (Wash Buffer 2)	4 ml	20 ml	100 ml	RT
Elution Buffer	2 ml	10 ml	5 x 10 ml	RT
DNA Purification Columns	10 pcs	50 pcs	5 x 50 pcs	RT
Collection Tubes (2 ml)	10 pcs	50 pcs	5 x 50 pcs	RT

[1] RT – room temperature (+15°C to +25°C

[2] After reconstitution, Proteinase K should be kept at -20°C.

[3] DTT solution should be stored at -20°C.

[*] Prior to the first use, add 320 µl Proteinase Buffer to a tube containing Proteinase K lyophilizate (in the kit for 10 isolations 200 µl of a buffer should be added).

[**] Prior to the first use, dilute the DTT in sterile water, following instructions on the vial label.

In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing.

Expiry date

Under proper storage conditions the kit will remain stable for at least 12 months from opening or until the expiry date.

III. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- sterile swab sticks
- 96-100% ethanol PFA
- 1.5-2ml sterile microcentrifuge tubes
- automatic pipettes and pipette tips
- disposable gloves
- microcentrifuge with rotor for 1.5-2 ml (≥ 11 000 x g)
- dry block heater or water bath (up to 70°C)
- vortex mixer

Might be necessary:

- RNase A
- PBS buffer

IV. PRINCIPLE

DNA purification procedure consists of four steps and utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids. Swab or semen sample is subjected to enzymatic lysis by Proteinase K in SSL Buffer. When isolating from semen, DTT must also be used. In this step, cell walls, membranes and proteins are degraded by lysis buffer and Proteinase K. After addition of chaotropic salts, lysate is applied to purification minicolumn membrane and DNA is bound. A two-step washing stage effectively removes impurities and enzyme inhibitors. Purified DNA is eluted with then use of a low ionic strength buffer (Elution Buffer) or water (pH 7.0-9.0) and can be used directly in all downstream applications such as PCR, qPCR, Southern blotting, DNA sequencing, enzymatic restriction, DNA ligation and so forth, or stored until ready to use.

V. QUALITY CONTROL

The quality of each production batch (LOT) of the ***EXTRACTME*** **DNA SWAB & SEMEN KIT** is tested using standard QC procedures. Purified DNA concentration and quality are evaluated by gel electrophoresis and spectrophotometer.

VI. PRODUCT SPECIFICATIONS

SAMPLE MATERIAL
Buccal, nasal, pharyngeal, vaginal, blood and saliva swabs or semen.
EFFICIENCY
Dependent on the sample material amount and type. Up to 3 µg DNA from a swab and 2-7 µg from 150 µl of a semen sample.
BINDING CAPACITY
Approx. 25 µg DNA
TIME REQUIRED
45-50 minutes (including incubation time)
DNA PURITY
A_{260}/A_{280} ratio = 1.7 – 1.9

VII. SAFETY PRECAUTIONS

- Swabs and semen samples are treated as biohazardous materials of its potential pathogen content or health and life-threatening substances. While working with swabs/semen, compliance with it is essential to comply with all safety requirements for working with biohazardous material.
- It is advisable to conduct the entire isolation procedure in a Class II Biological Safety Cabinet or at laboratory burner as well as wearing disposable gloves and suitable lab coat at all times.
- The use of sterile filter tips is recommended.
- Avoid the cross-contamination of DNA between minicolumns.
- Guanidine salts' residues may form highly reactive compounds when combined with oxidation components. In case of spillage, clean the surface with detergent-water solution.

VIII. RECOMMENDATIONS AND IMPORTANT NOTES

DNA elution

Optimal volume of Elution Buffer used should be chosen in line with type and amount of sample material and final DNA concentration expected. The use of 50-100 µl of Elution Buffer is recommended.

Quantity of purified DNA depends on type of sample and number of cells it contains, in other words, quality of swab, features of site swab was taken from and interindividual diversity. Usually, isolation efficiency from one buccal swab is 1-3 µg of DNA and from 150 µl of semen, 2-7 µg.

If a high DNA concentration is desired, elution volume may be reduced to 20 µl. It should be noted that this may reduce efficiency of DNA retrieval. It is essential to apply Elution Buffer precisely to the centre of membrane.

In order to maximize DNA retrieval, heat Elution Buffer to 80°C and incubate it on membrane for 10 minutes.

If full DNA retrieval is required, a second elution should be performed. For second elution, repeat steps 20-23 of the Isolation Protocol (section XI), placing purification column in a new, sterile 1.5 ml Eppendorf tube.

Elution Buffer does not contain EDTA, which may interfere with some enzymatic reactions. If desired, DNA can alternatively be eluted from minicolumn with nuclease-free water (pH 7.0-9.0) or TE Buffer.

RNA contamination

Co-extracted vestigial RNA contamination may interfere with some enzymatic reaction, but does not inhibit PCR. If an RNA-free DNA sample is desired, add 4 µl of the RNase A solution (10 mg/ml; cat no. RP14, RP45) to SSL Buffer in step 2 of the Isolation Protocol. Mix well and incubate at 37°C for 5 minutes. After incubation, add Proteinase K and DTT when isolating from semen and continue isolation following the Isolation Protocol (section XI).

IX. SAMPLE PREPARATION

A. SEMEN

Sample of semen into a sterile container. The kit is designed for isolation from both freshly sampled semen and a frozen sample. Semen can be stored at +4°C for a short time or frozen (-80°C is highly recommended) for a longer period. Avoid subjecting material to repeated freeze/thaw cycles before isolation. Before sampling, mix material thoroughly.
If sample volume is less than 150 µl, add Elution Buffer or PBS buffer (not included in the kit) up to 150 µl and follow the Isolation Protocol from step 1 (section XIA). It should be noted that efficiency of DNA isolation will be lower.

B. SWABS

To collect a buccal swab, scrape a sterile swab stick firmly against inside of cheek at least 10 times. Place swab holding tissue cells in a 1.5-2 ml Eppendorf tube and cut off excess from the end of shaft. It is not necessary to dry the swab. Ensure that person providing sample has not consumed any food or drink during the 30 minutes prior to sample collection. Using low quality material considerably reduces DNA isolation efficiency. Sample can be collected using any commercially available buccal swab stick. A swab sample can be stored at +4°C for a short time or frozen for a longer period (-80°C is highly recommended) for longer period of time.

X. PRIOR TO ISOLATION

1. Mix well each buffer supplied with the kit.
2. Prepare **Proteinase K** solution by reconstituting lyophilizate in an appropriate quantity of Proteinase Buffer. Reconstitute **DTT** powder in water, following instructions on vial label.
3. Examine buffers. If a sediment has occurred in any of buffers, incubate it at 37°C (**SSW1** and **SSW2** Buffers) or at 50-60°C (other buffers), mixing occasionally until the sediment has dissolved. Cool to room temperature.
4. Set a dry block heater or water bath to 56°C.
5. Unless otherwise stated, conduct all isolation steps at room temperature.

XI. ISOLATION PROTOCOL

A. SEMEN

1. Transfer **150 µl semen** to a sterile, 1.5-2 ml Eppendorf tube.
   ⚠ The sampling method is described in section IXA. Sample preparation.
2. Add **350 µl SSL Buffer**, **6 µl Proteinase K** and **20 µl 1M DTT**, vortex for 3 s.
3. Follow the Isolation Protocol from step 3 (section XIB).

B. SWABS

1. Place a buccal swab holding material in a 1.5 ml Eppendorf tube and cut off excess from the end of shaft so that tube lid can be closed without difficulty.
   ⚠ The sampling method is described in section IXB. Sample preparation.
2. Add **350 µl SSL Buffer** and **6 µl Proteinase K**, vortex for 3 s.
3. Incubate at **56°C** for 30 min. Mix by inverting tube at several-minute intervals during the incubation.
4. Add **350 µl SSB Buffer** and mix thoroughly.
5. Incubate for an additional 6 min at **70°C**.
6. Press swab firmly against one side of tube in order to retrieve maximum possible volume of lysate. Discard swab.
   ⚠ Lysate can be transferred to purification minicolumn without retrieving it from swab. However, isolation efficiency will be reduced.
7. Add **200 µl 96-100% ethanol** (not included in the kit) and mix well by inverting tube several times.
8. Transfer **700 µl** of **lysate** onto a purification minicolumn placed in a collection tube.

9. Centrifuge for 60 s at 11 000-15 000 x g.
10. Discard filtrate and reuse collection tube.
11. Transfer all of remaining lysate onto purification column.
12. Centrifuge for 60 s at 11 000-15 000 x g.
13. Transfer purification minicolumn to a new collection tube.
14. Add **600 µl SSW1 Buffer** and centrifuge for 30 s at 11 000-15 000 x g.
15. Discard filtrate and reuse collection tube.
16. Add **400 µl SSW2 Buffer** and centrifuge for 30 s at 11 000-15 000 x g.
17. Discard filtrate and reuse the collection tube.
18. Centrifuge for 60-120 s at 15 000-21 000 x g.
    ⚠ Wash buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease elution efficiency. It is therefore vital to remove alcohol completely from minicolumn prior to the elution.
19. Discard collection tube and filtrate and carefully transfer purification minicolumn to a sterile, 1.5 ml Eppendorf microcentrifuge tube.
20. Add **50-100 µl Elution Buffer**, directly onto purification minicolumn membrane.
    ⚠ Other buffer volumes between 20-100 µl range may be used. For instructions, see section VIII. Recommendations and important notes.
21. Incubate minicolumn at room temperature for 120 s.
22. Centrifuge at 11 000-15 000 x g for 60 s.
23. Remove minicolumn. Isolated DNA should be stored at **+4°C** or **-20°C** depending on further applications.

XII. TROUBLESHOOTING

Problem	Possible cause	Solution
Column becomes clogged during purification.	Swab sample contained food remains.	Repeat isolation, ensuring that person providing sample does not consumed any food or drink for 30 minutes prior to sample collection (refer to section IXB. Sample preparation).
Low yield of purified DNA.	Improper sample collection method. Swab contains too few peeling cells.	When collecting a sample, ensure that swab stick is scraped firmly against inside of cheek (refer to IXB. Sample preparation).
	Semen was collected from a male with oligospermia.	Use more semen material, increasing the volume of relevant solutions (SSL Buffer, Proteinase K, DTT, SSB Buffer) and of ethanol. Apply 700 µl of lysate onto a minicolumn and repeat as necessary. After each application, centrifuge minicolumn at 11 000-15 000 x g for 60 s and discard supernatant.
	Incomplete cell lysis.	Extend incubation time at 56°C. Mix by inverting at several-minute intervals.
	The purified DNA contains residual alcohol.	Repeat the isolation, giving particular attention to ensuring that no residual SSW2 Buffer is left in the purification minicolumn after centrifugation in step 18.
	Incomplete DNA elution from the membrane.	Before applying Elution Buffer to membrane, heat it to 80°C. Apply Elution Buffer directly to centre of membrane. Extend incubation time with Elution Buffer to 10 min. Perform second elution. Increase volume of Elution Buffer to 200 µl.
	pH of the water used for elution is lower than 7.0.	Use Elution Buffer for DNA elution.
Isolated DNA is of poor purity.	Swab sample is of very low purity.	Use 600 µl of SSW2 Buffer during second washing step (step 16 of Isolation Protocol), and centrifuge at 11 000-15 000 x g for 60 s. Empty collection tube and re-spin dry minicolumn (step 18 of the Isolation Protocol). Ensure that person providing sample does not consume any food or drink during 30 min prior to sample collection.
	Incomplete protein digestion as a result of reduced Proteinase K activity.	Prepare a fresh Proteinase K solution. Make sure Proteinase K solution is stored at -20°C.
	One of washing steps was omitted.	Repeat isolation, performing both washing steps.
	Purified DNA contains residual alcohol.	Repeat isolation, giving particular attention to ensuring that no residual SSW2 Buffer is left in purification column after centrifugation in step 18.

Purified DNA is degraded.	Inappropriate sample storage conditions.	Storing swabs and semen samples at -80°C is recommended. Avoid subjecting sample material to repeated freeze/thaw cycles.
RNA contamination present.	Incubation with RNase A was too short.	Extend incubation time with RNase A to 30 min (step 4 of the Isolation Protocol).
	Reduced RNase A activity.	Prepare a fresh RNase A solution and repeat isolation. Ensure proper storage conditions of RNase A solution: +4°C for short-term storage and -20°C for long-term.

XIII. SAFETY INFORMATION

SSL Buffer

Warning
H319
P264, P305+P351+P338

SSB Buffer

Danger
H302, H315, H318, H332, H412
P261, P280, P301+P312 P330, P304+P340 P312,
P305+P351+P338 P310

Proteinase K

Danger
H315, H319, H334, H335
P261, P271, P304+P340, P342+P311, EUH208

DTT

Warning
H302, H315, H319, H335
P261, P301+P312 P330, P304+P340

SSW1 Buffer

Danger
H225, H315, H319, H336
P210, P304+P340 P312, P305+P351+P338

SSW2 Buffer

Danger
H225, H319, H336
P210, P261, P305+P351+P338

EUH208 Contains Proteinase. May produce an allergic reaction. **H225** Highly flammable liquid and vapour. **H302** Harmful if swallowed. **H315** Causes skin irritation. **H318** Causes serious eye damage. **H319** Causes serious eye irritation. **H332** Harmful if inhaled. **H334** May cause allergy or asthma symptoms or breathing difficulties if inhaled. **H335** May cause respiratory irritation. **H336** May cause drowsiness or dizziness. **H412** Harmful to aquatic life with long-lasting effects. **P210** Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. **P261** Avoid breathing dust/fumes/gas/mist/vapours/spray. **P264** Wash hands thoroughly after handling. **P271** Use only outdoors or in a well-ventilated area. **P280** Wear protective gloves/protective clothing/eye protection/face protection. **P305+P351+P338** IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. **P310** Immediately call a POISON CENTER / doctor. **P301+P312 P330** IF SWALLOWED: call a POISON CENTER / doctor if you feel unwell. Rinse mouth. **P304+P340** IF INHALED: remove person to fresh air and keep comfortable for breathing. **P312** Call a POISON CENTER / doctor if you feel unwell. **P342+P311** If experiencing respiratory symptoms: call a POISON CENTER or doctor/physician.