mawi

# DNA extraction Protocol for iSWAB-DNA using QIAamp® DNA Mini kit (or DNeasy® Blood & Tissue)

## Kits

QIAamp® DNA Mini and Blood Mini kit (Cat No 51104)
DNeasy® Blood & Tissue kit (Cat No 69504)

## Additional equipment and reagents required

1. Nuclease free water
2. Microcentrifuge
3. PBS buffer pH 7.4

## Important points before starting

1. All centrifugation steps are carried out at room temperature (15–25°C).

## Things to do before starting

1. Heat a water bath or heating block to 56°C.

2. Equilibrate Buffer AE or distilled water to room temperature (15–25°C) for elution.

3. Ensure that Buffer AW1, Buffer AW2, and QIAGEN Protease have been prepared according to the instructions below
   i) Buffer AW1 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle.
   ii) Buffer AW1 is stable for 1 year when stored closed at room temperature.
   iii) Buffer AW2 is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) to Buffer AW2 concentrate as indicated on the bottle.
   iv) Buffer AW2 is stable for 1 year when stored closed at room temperature

4. If a precipitate has formed in Buffer AL, dissolve by incubating at 56°C.Buffer AL is stable for 1 year when stored at room temperature.

## Protocol

1. Pipette 200-300µl from iSWAB collected sample into a clean labeled 1.5ml microcentrifuge tube. In case of 1mL inputs, you can adjust the volume of the sample to 1mL in nuclease free water or PBS buffer (pH 7.4).

If the sample volume is > 200µl then split the sample into two 1.5ml microcentrifuge tubes, with the final volume adjusted to 200µl in each tube. Then each of the 200µl sample is treated similarly.

2. Add 200µl **Buffer AL** to the sample. Mix by pulse-vortexing for 15 s.

   - To ensure efficient lysis, it is essential that the sample and Buffer AL are mixed thoroughly to yield a homogeneous solution. If the sample volume is larger than 200µl, increase the amount of QIAGEN Protease (or proteinase K) and Buffer AL proportionally; for example, a 400µl sample will require 40µl QIAGEN Protease (or proteinase K) and 400µl Buffer AL.
     **Note**: Do not add QIAGEN Protease or proteinase K directly to Buffer AL.

3. Incubate at 56ºC for 10 min.

4. Add 200µl **ethanol** (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5ml microcentrifuge tube to remove drops from the inside of the lid.

   - If the sample volume is greater than 200µl, increase the amount of ethanol proportionally; for example, a 400µl sample will require 400µl of ethanol.

5. Carefully apply the mixture from step 6 to the QIAamp Mini spin column (in a 2ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (11,000 rpm) for 2 min. Discard the collection tube containing the filtrate.

   - If the starting amount in step 2 is 400µl (split into two 200µl tubes), then repeat step 8 by applying the second mixture to the same QIAamp Mini spin column and centrifuge at 6000 x g (11000 rpm) for 2 min.
     **Note:** If the lysate has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp Mini spin column is empty.

6. Place the QIAamp Mini spin column in a clean 2ml collection tube (provided).Carefully open the QIAamp Mini spin column and add 500µl of **Buffer AW1** without wetting the rim. Close the cap and centrifuge at 6000 x g (11,000 rpm) for 1 min. Discard the collection tube containing the filtrate.

   - It is not necessary to increase the volume of Buffer AW1 if the original sample volume is larger than 200µl.

7. Place the QIAamp Mini spin column in a clean 2ml collection tube (provided).Carefully open the QIAamp Mini spin column and add 500µl **Buffer AW2** without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min. Discard the collection tube containing the filtrate.

8. Place the QIAamp Mini spin column in a new 2ml collection tube (not provided). Centrifuge at full speed for 1 min. Discard the collection tube containing any filtrate.

    - This step helps to eliminate the chance of possible Buffer AW2 carryover.

**9.** Place the QIAamp Mini spin column in a clean labelled 1.5ml microcentrifuge tube with a lid cut off (not provided). Carefully open the QIAamp Mini spin column and add 150µl **Buffer AE** or **Ultrapure water**. Incubate at room temperature (15–25°C) for 5 min, and then centrifuge at 6000 x g (11000 rpm) for 1 min.

**Note: Performing a heated elution step may increase yield. Add 150µl Elution Buffer (preheated to 65°C) and incubate at room temperature for 5 min. Also, performing a re-elution step can help release more DNA from the columns and thus increasing the yield.**

- **Note:** Volumes of more than 200µl should not be eluted into a 1.5ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation.

10. Transfer the DNA sample to a freshly labeled 1.5ml microcentrifuge tube and nanodrop the sample. Note the DNA concentration on the 1.5ml microcentrifuge tube as ng/µl.