A&A BIOTECHNOLOGY
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Manual

Sherlock AX

Kit for DNA purification from materials with trace content of DNA (blood and saliva stains, hair, fur, tissue preserved in paraffin and formalin, fresh tissue, fresh and frozen blood).
Procedure with DNA precipitation.

catalog #	size
095-25	25 isolations
095-100	100 isolations

For research use only.

Guarantee
A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

Contents

component	095-25	095-100	storage
Spin 10AX columns	25 pcs	100 pcs	2–8 °C
Filter 1 filtration columns	25 pcs	100 pcs	15–25 °C
2 ml tubes	50 pcs	200 pcs	15–25 °C
L1.4 lysis solution	9 ml	36 ml	15–25 °C
K2 wash solution	40 ml	160 ml	15–25 °C
K3 elution solution	23 ml	92 ml	15–25 °C
Precipitation enhancer	300 µl	1.2 ml	15–25 °C
TE buffer	1.5 ml	5 ml	15–25 °C
Isopropanol	20 ml	80 ml	15–25 °C
Proteinase K	600 µl	2 x 1.1 ml	2–8 °C

The binding capacity of the minicolumn is 10 µg.

Additional equipment and reagents

Necessary

- 1.5 ml sterile Eppendorf tubes
- Sterile water (cat. # 003-075, 003-25)
- 1M DTT (cat. # 2010-5, 2010-25, 2010-10P)
- 70% ethanol
- Hexane / xylene / 96% ethanol (for paraffin embedded tissue)
- Vortex
- Microcentrifuge
- Incubator or thermoblock set to 50 °C

Optional

- Tris buffer (10 mM, pH 8.0)

Material preparation

Forensic samples

1. Transfer **dried sample (**blood, saliva, sperm) to a 1.5 ml tube (not included).
2. Add:
   300 µl of **sterile water** (not included),
   300 µl of **L1.4** lysis solution,
   20 µl of **proteinase K**.

   For sperm samples: add **20 µl** of **1M DTT** (not included).
3. Vortex for **20 s**.
4. Incubate for **60 min** at **50 °C**. Vortex the sample from time to time.
5. Follow point 1. of the isolation protocol.

Blood (fresh or frozen)

1. Transfer **300 µl** of blood to a 1.5 ml tube (not included).

   Note: for blood volume less than 300 µl, add sterile water to a total volume of 300 µl.
2. Add **300 µl** of **L1.4** lysis solution and **20 µl** of **proteinase K**.
3. Vortex for **20 s**.
4. Incubate for **10 min** at **50 °C**. Vortex the sample from time to time.
5. Follow point 1. of the isolation protocol.

Fresh tissues

1. Transfer up to **10-20 mg** of **fragmented tissue** to a 1.5 ml tube (not included).
2. Add:
   300 µl of **sterile water** (not included),
   300 µl of **L1.4** lysis solution,
   20 µl of **proteinase K**.
3. Vortex for **20 s**.
4. Incubate for **1-2 h** at **50 °C**. Vortex the sample from time to time.
5. Follow point 1. of the isolation protocol.

Paraffin embedded tissues

1. Transfer the **tissue in a paraffin block** to a 1.5 ml tube (not included).
2. Add the appropriate amount of **xylene** or **hexane** (not included) to immerse the sample completely.
3. Mix the sample by inverting the tube and wait to dissolve the visible wax.
   Centrifuge for **20 s** at **10 000 RPM**, discard the supernatant. Repeat dewaxing 2-3 times.
4. Remove residual hexane / xylene by washing twice with **96% ethanol** (not included).
   Remove residual ethanol by keeping the tissue sample for **2-5 min** at **room temp**.
5. Follow point 2. of the material preparation protocol - fresh tissues.

Formalin fixed tissues

1. Transfer the **tissue** to a 1.5 ml tube (not included).
2. Add the appropriate amount of **xylene** or **hexane** (not included) to immerse the sample completely.
3. Mix the sample by inverting the tube.
   Centrifuge for **20 s** at **10 000 RPM**, discard the supernatant. Repeat 3-4 times.
4. Follow point 2. of the material preparation protocol - fresh tissues.

Hair, fur

1. Cut **hair, fu**r into **small ~0.5 pieces** and transfer to a 1.5 ml tube (not included).
2. Add:
   300 µl of **sterile water** (not included),
   300 µl of **L1.4** lysis solution,
   20 µl of **proteinase K**,
   20 µl of **1M DTT** (not included).
3. Vortex for **20 s**.
4. Incubate at **50 °C** until completely dissolved. Vortex the sample from time to time.
5. Follow point 1. of the isolation protocol.

Protocol

1.	Apply the samples onto the **Filter 1** filtration columns.
2.	Centrifuge for **1 min** at **10 000 RPM (9000 x g)**.
3.	Remove the **Filter 1** filtration columns. Apply the filtrates with DNA onto the **Spin 10AX** columns.
4.	Centrifuge for **1 min** at **8000 RPM (6000 x g)**.
5.	Discard the filtrates. Place the Spin 10AX columns into **new 2 ml** tubes (included).
6.	Add **600 µl** of **K2** wash solution.
7.	Centrifuge for **1 min** at **8000 RPM (6000 x g)**.
8.	Discard the filtrates. Place the Spin 10AX columns into **the same** tubes.
9.	Add **600 µl** of **K2** wash solution.
10.	Centrifuge for **1 min** at **8000 RPM (6000 x g)**.
11.	Discard the filtrates. Place the Spin 10AX columns into **new 2 ml** tubes (included).
12.	Add **350 µl** of **K3** elution solution.
13.	Keep the samples for **2 min** at **room temp.**
14.	Centrifuge for **1 min** at **8000 RPM (6000 x g)**.
15.	Add **350 µl** of **K3** elution solution.
16.	Keep the samples for **1 min** at **room temp.**
17.	Centrifuge for **1 min** at **8000 RPM (6000 x g)**.

18. Remove the Spin 10AX columns.
Transfer the filtrates with DNA (**~700 µl**) to **new 1.5 ml** tubes (not included).

19. Add **5 µl** of **precipitation enhancer** and **600 µl** of **isopropanol**.

20. Mix the samples by inverting the tubes a few times and centrifuge for **10 min** at **10 000 RPM (9000 x g)**.

21. Carefully discard supernatant. Be careful not to remove the DNA pellet at the bottom of the tube.

Note. The light-blue DNA pellet should be visible at the bottom of the precipitation tube.

22. Add **500 µl** of **70% ethanol** (not included).

23. Mix the sample and centrifuge for **5 min** at **10 000 RPM (9000 x g)**.

24. Carefully discard supernatant. Air dry the DNA pellet for **10 min** at **room temp**. up-site down.

Note. If there are any leftovers (small droplets) of alcohol on the tube walls they should be removed with sterile cotton buds.

25. Dried DNA pellets can be dissolved in **TE** buffer (included), sterile water (not included) or 10 mM Tris buffer, pH 8.0 (not included).

26. Store the purified DNA at 4 °C until later use.

Safety Information

DANGER

Proteinase K

H315 Causes skin irritation.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335 May cause respiratory irritation.
P261 Avoid breathing dust.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P342+P311 If experiencing respiratory symptoms call a Poison Center or doctor/physician.

WARNING

L1.4 lysis solution

H302 Harmful if swallowed.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

K2 wash solution

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

K3 elution solution

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

DANGER

Isopropanol

H225 Highly flammable liquid and vapor.
H319 Causes serious eye irritation.
H336 May cause drowsiness or dizziness.
P210 Keep away from heat, sparks, open flames, hot surfaces. No smoking.
P261 Avoid breathing vapors.
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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