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.
version 0517

25 isolations, 100 isolations
Cat. # 095–25, 095–100

The binding capacity of the DNA purification column is 10 µg of DNA.

For R&D use only.
Kit Contents

<table>
<thead>
<tr>
<th>Component</th>
<th>25 isolations</th>
<th>100 isolations</th>
<th>Store at</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columns Spin 10 AX (blue ring)</td>
<td>25 pcs</td>
<td>100 pcs</td>
<td>+4 to +8 ºC</td>
</tr>
<tr>
<td>Filtration columns Filter 1 (yellow ring)</td>
<td>25 pcs</td>
<td>100 pcs</td>
<td>Room Temp.</td>
</tr>
<tr>
<td>2 ml tubes</td>
<td>25 pcs</td>
<td>100 pcs</td>
<td>Room Temp.</td>
</tr>
<tr>
<td>L1.4 lysis solution</td>
<td>9 ml</td>
<td>36 ml</td>
<td>Room Temp.</td>
</tr>
<tr>
<td>K2 wash solution</td>
<td>40 ml</td>
<td>160 ml</td>
<td>Room Temp.</td>
</tr>
<tr>
<td>K3 elution solution</td>
<td>23 ml</td>
<td>92 ml</td>
<td>Room Temp.</td>
</tr>
<tr>
<td>Precipitation enhancer</td>
<td>300 µl</td>
<td>1.2 ml</td>
<td>Room Temp.</td>
</tr>
<tr>
<td>TE buffer</td>
<td>1.5 ml</td>
<td>5 ml</td>
<td>Room Temp.</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>600 µl</td>
<td>2 x 1.1 ml</td>
<td>+4 to +8 ºC</td>
</tr>
<tr>
<td>I solution</td>
<td>20 ml</td>
<td>80 ml</td>
<td>Room Temp.</td>
</tr>
</tbody>
</table>

Equipment and materials necessary for the isolation DNA that not included in kit

1. Material for DNA isolation
2. Sterile water (nuclease free, DEPC treated) (cat. # 003-075, 003-25)
3. DTT (dithiothreitol) for sperm, hair, fur samples only (cat. # 2010-5, 2010-25)
4. 70% ethanol
5. 96% ethanol (for paraffin embedded tissue only)
6. Hexane / xylene (for paraffin embedded tissue only)
7. 1.5 ml sterile Eppendorf tubes
8. Vortex
9. Benchtop microcentrifuge
10. Heatblock or incubator set to 50 ºC

NOTE:
Before you start working, we recommend cleaning the work surface using LabZAP™ product (cat. # 040-500)

A&A Biotechnology provides one year guarantee on this kit
The company does not guarantee correct performance of this kit in the event of:
- not adhering to the supplied protocol
- not recommended use of equipment and materials
- the use of other reagents than recommended or which are not a component of the kit
- the use of expired or improperly stored reagents and columns
Material preparation

Forensic samples
1. Transfer dried sample (blood, saliva, sperm) into an Eppendorf tube (not included).
2. Add:
   - 300 µl of sterile water (not included, cat. # 003–075, 003–25),
   - 300 µl of L 1.4 lysis solution,
   - 20 µl of Proteinase K,
   - 20 µl of 1M DTT (for sperm samples only)
     (not included, cat. # 2010–5, 2010–25).
3. Mix by vortexing for 20 s.
4. Incubate for 60 min at 50 °C (mix from time to time).
5. Follow point 1. of the isolation protocol.

Blood (fresh or frozen)
1. Transfer 300 µl of blood (if less, add sterile water up to total 300 µl volume) into an Eppendorf tube (not included).
2. Add 300 µl of L 1.4 lysis solution and 20 µl of Proteinase K.
3. Mix by vortexing for 20 s.
4. Incubate for 10 min at 50 °C (mix from time to time).
5. Follow point 1. of the isolation protocol.

Fresh tissues
1. Cut 10–20 mg of tissue to small pieces and transfer into an Eppendorf tube (not included).
2. Add:
   - 300 µl of sterile water (not included, cat. # 003–075, 003–25),
   - 300 µl of L 1.4 lysis solution
   - 20 µl of Proteinase K.
3. Mix by vortexing for 20 s.
4. Incubate for 120–240 min at 50 °C (mix from time to time).
5. Follow point 1. of the isolation protocol.

Paraffin embedded tissues
1. Remove the paraffin from the tissue block by adding the appropriate amount of xylene or hexane to immerse the sample completely. Mix the sample by inverting the tube and wait to dissolve the visible wax. Spin down the tube for 20 s at 10 000 RPM and remove supernatant. Repeat deparaffinizing 2–3 times.
2. After deparaffinizing remove the residual hexane/xylene by double washing the sample with 96% ethanol. Remove the residual of ethanol by leaving the tissue sample for 2–5 min at room temp.
3. Follow point 2. of the material preparation protocol – fresh tissues.
**Formalin fixed tissues**
1. Transfer tissue into an Eppendorf tube (not included).
2. Add the appropriate volume of sterile water (not included, cat. # 003–075, 003–25) to immerse the sample completely. Mix the sample by inverting the tube. Spin down the tube and remove the supernatant. Wash 3–4 times.
3. Follow point 2. of the material preparation protocol – fresh tissues.

**Hair, fur**
1. Cut hair, fur into small pieces ~0.5 cm, transfer into an Eppendorf tube (not included).
2. Add:
   - 300 µl of sterile water (not included, cat. # 003–075, 003–25),
   - 300 µl of L 1.4 lysis solution,
   - 20 µl of Proteinase K,
   - 20 µl of 1M DTT (not included, cat. # 2010–5, 2010–25).
3. Mix by vortexing for 20 s.
4. Incubate at 50 °C until completely dissolved (mix from time to time).
5. Follow point 1. of the isolation protocol.

**Isolation protocol**

1. Apply the samples onto filtration columns Filter 1 (yellow ring).

2. Centrifuge for 1 min at 10 000 RPM (9000 x g).

3. Discard the filtration columns Filter 1. Apply the filtrates with DNA onto the Spin 10 AX (blue ring) columns.

4. Centrifuge for 1 min at 8000 RPM (6000 x g).

5. Discard the filtrates from the tubes and re-attach the Spin 10 AX columns.
6. Add 600 µl of K2 wash solution. Centrifuge for 1 min at 8000 RPM (6000 x g).

7. Discard the filtrates from the tubes and re-attach the Spin 10 AX columns.

8. Add 600 µl of K2 wash solution. Centrifuge for 1 min at 8000 RPM (6000 x g).

9. Discard the filtrates from the tubes and transfer the Spin 10 AX columns into new 2 ml tubes (included).

10. Apply 350 µl of K3 elution solution onto the Spin 10 AX columns. Incubate for 2 min at room temp.

11. Centrifuge for 1 min at 8000 RPM (6000 x g).

12. Apply 350 µl of K3 elution solution onto the Spin 10 AX columns. Incubate for 1 min at room temp.

13. Centrifuge for 1 min at 8000 RPM (6000 x g).

14. Remove the Spin 10 AX columns. Transfer the filtrates with DNA (~ 700 µl) into new 1.5 ml tubes (not included).
15. Add 5 µl of precipitation enhancer and 600 µl of I solution. Thoroughly mix by inverting the tubes. Centrifuge for 10 min at 10 000 RPM (9000 x g).

16. Carefully remove the supernatants. The light blue pellet should be visible at the bottom of the tube.

17. Add 500 µl of 70% ethanol (not included). Mix and centrifuge for 5 min at 10 000 RPM (9000 x g).

18. Carefully remove the supernatants. Air dry the pellets for 10 min at room temp.

19. Suspend the pellets in TE buffer (included), sterile water (not included, cat. # 003–075, 003–25) or Tris buffer 10 mM, pH 8.0 (not included). The blue colour of DNA precipitate enables visual confirmation of the DNA dissolution process.

20. Store purified DNA at 4 ºC until later use.
## Kit Specification

<table>
<thead>
<tr>
<th>Material</th>
<th>anion exchange membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal sample volume</td>
<td></td>
</tr>
<tr>
<td>blood</td>
<td>300 µl</td>
</tr>
<tr>
<td>cell culture</td>
<td>$10^6$</td>
</tr>
<tr>
<td>bacterial culture</td>
<td>$10^9$</td>
</tr>
<tr>
<td>tissue</td>
<td>20 mg</td>
</tr>
<tr>
<td>Binding capacity</td>
<td>10 µg</td>
</tr>
<tr>
<td>Operate</td>
<td>spin, vacuum</td>
</tr>
<tr>
<td>Elution solution</td>
<td>high salt, precipitation with linear polyacrylamide carrier</td>
</tr>
<tr>
<td>Minimum elution volume</td>
<td>100 µl</td>
</tr>
<tr>
<td>Advantages</td>
<td>95% of DNA recovery</td>
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<tr>
<td></td>
<td>highest purity</td>
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<tr>
<td></td>
<td>Excellent for forensic science</td>
</tr>
<tr>
<td>Applications</td>
<td>PCR, southern blotting, gene library cloning</td>
</tr>
</tbody>
</table>

## Ordering information

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinase K solution (20 mg/ml)</td>
<td>1.1 ml</td>
<td>1019–20</td>
</tr>
<tr>
<td>Proteinase K lyophilized</td>
<td>25 mg</td>
<td>1019–25L</td>
</tr>
<tr>
<td></td>
<td>100 mg</td>
<td>1019–100L</td>
</tr>
<tr>
<td></td>
<td>250 mg</td>
<td>1019–250L</td>
</tr>
<tr>
<td></td>
<td>1000 mg</td>
<td>1019–1L</td>
</tr>
<tr>
<td>DTT (dithiothreitol)</td>
<td>5 g</td>
<td>2010–5</td>
</tr>
<tr>
<td></td>
<td>25 g</td>
<td>2010–25</td>
</tr>
</tbody>
</table>
**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 vapour.
- 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 vapours.
- 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 vapour.
- 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 vapours.
- 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**

**I solution**
- H225 Highly flammable liquid and vapour.
- 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 vapours.
- P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.