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Extraction of DNA from endocervical samples using QIAamp DNA mini kit

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ABSTRACT

DNA was extracted from endocervical samples using the QIAamp mini kit according to the manufacturer's instructions.

1 Centrifuge tubes containing the endocervical specimens at 15,000 rpm for

🕒 00:10:00 .

2 Remove supernatant and preserve the pellet;

3 Add 🧴 180 µL of the ATL buffer and homogenize with the help of a vortex mixer.

4 Add 🧴 20 µL Proteinase K to the homogenized mixture and mix by vortexing

5 Incubate the solution in a dry bath at 🔥 56 °C for 🕒 03:00:00 while homogenizing 2 to 3 times during incubation.

6 Add 200 µL AL of buffer and homogenize again using a vortex for 🕒 00:00:15

7 Incubate the solution in a dry bath at 🔥 70 °C for 🕒 00:10:00 .

8 Add 🧴 200 µL of ethanol and homogenize.


9 Then transfer the mixture carefully into a QIAamp mini spin column and centrifuge at 8,000 rpm for 🕒 00:01:00 .


10 Discard filtrate and place the minicolumns were placed QIAamp mini spin column in new 2 mL tubes


11 Add of  **500 µL** buffer AW1, centrifuged at 8,000 rpm for  **00:01:00** and discard the filtrate.

12 Add of  **500 µL** buffer AW2 was added to minicolumns.

13 Centrifuge at 14,000 rpm for 3 minutes and discard the filtrate.

14 Centrifuge the spin column with the collection tube again at 14,000rpm for  **00:01:00**

15 Place minicolumns in 1.5 mL micro-centrifuge tubes and add  **50 µL** of buffer AE.

16 Incubate at room temperature for 5 minutes and centrifuge the mixture at 10,000 rpm for  **00:03:00** .

17 Discard the QIAamp mini spin column and store micro-centrifuge at -20 °C for subsequent molecular analysis.

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6 Add 200 µL AL of buffer and homogenize again using a vortex for ⌚00:00:15

7 Incubate the solution in a dry bath at 🌡70 °C for ⌚00:10:00 .

8 Add 📏200 µL of ethanol and homogenize.

9 Then transfer the mixture carefully into a QIAamp mini spin column and centrifuge at 8,000 rpm for ⌚00:01:00 .

10 Discard filtrate and place the minicolumns were placed QIAamp mini spin column in new 2 mL tubes

11 Add of 📏500 µL buffer AW1, centrifuged at 8,000 rpm for ⌚00:01:00 and discard the filtrate.

12 Add of 📏500 µL buffer AW2 was added to minicolumns.

13 Centrifuge at 14,000 rpm for 3 minutes and discard the filtrate.

14 Centrifuge the spin column with the collection tube again at 14,000rpm for ⌚00:01:00

15 Place minicolumns in 1.5 mL micro-centrifuge tubes and add 📏50 µL of buffer AE.

16 Incubate at room temperature for 5 minutes and centrifuge the mixture at 10,000 rpm for ⌚00:03:00 .

17 Discard the QIAamp mini spin column and store micro-centrifuge at -20 °C for subsequent molecular analysis.

DNA was extracted from endocervical samples using the QIAamp mini kit according to the manufacturer's instructions.

- 1 Centrifuge tubes containing the endocervical specimens at 15,000 rpm for 10 minutes.
- 2 Remove supernatant and preserve the pellet;
- 3 Add 180 µL of the ATL buffer and homogenize with the help of a vortex mixer.
- 4 Add Proteinase K to the homogenized mixture and mix by vortexing
- 5 Incubate the solution in a dry bath at 56 °C for 3 hours while homogenizing 2 to 3 times during incubation.
- 6 Add 200 µL AL of buffer and homogenize again using a vortex for 15 seconds
- 7 Incubate the solution in a dry bath at 70°C for 10 minutes.
- 8 Add 200 µL of ethanol and homogenize.
- 9 Then transfer the mixture carefully into a QIAamp mini spin column and centrifuge at 8,000 rpm for one minute.
- 10 Discard filtrate and place the minicolumns were placed QIAamp mini spin column in new 2 mL tubes
- 11 Add of 500 µL buffer AW1, centrifuged at 8,000 rpm for one minute and discard the filtrate.
- 12 Add of 500 µL buffer AW2 was added to minicolumns.
- 13 Centrifuge at 14,000 rpm for 3 minutes and discard the filtrate.
- 14 Centrifuge the spin column with the collection tube again at 14,000rpm for 1 minute
- 15 Place minicolumns in 1.5 mL micro-centrifuge tubes and add 50 µL of buffer AE.
- 16 Incubate at room temperature for 5 minutes and centrifuge the mixture at 10,000 rpm for 3 minutes.
- 17 Discard the QIAamp mini spin column and store micro-centrifuge at -20 °C for subsequent molecular analysis.