

Jul 11, 2022

Extraction of DNA from endocervical samples using QIAamp DNA mini kit

DOI

<https://dx.doi.org/10.17504/protocols.io.btnvnme6>



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DOI: <https://dx.doi.org/10.17504/protocols.io.btnvnme6>

Document Citation: emmanuelgomseu EBDG Boris Emmanuel DJOUMSIE GOMSEU 2022. Extraction of DNA from endocervical samples using QIAamp DNA mini kit. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.btnvnme6>

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Created: March 26, 2021

Last Modified: July 11, 2022

Document Integer ID: 48565

Keywords: using qiaamp dna mini kit, qiaamp dna mini kit, extraction of dna, discard the qiaamp mini spin column, placed qiaamp mini spin column, qiaamp mini spin column, qiaamp mini kit, endocervical sample, incubate at room temperature, endocervical specimen, centrifuge tube, centrifuge the spin column, times during incubation, extraction, subsequent molecular analysis, incubation, dna, homogenized mixture

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 mixe 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.

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 μ L 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 μ L 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 μ L 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 μ L buffer AW1, centrifuged at 8,000 rpm for one minute and discard the filtrate.
- 12 Add of 500 μ L 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 μ L 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.