Lobsterpot
CA19138

Common SOPs for DNA/RNA/protein extraction, quantification and qualification as well as for the establishment of tissue microarrays (TMAs)

Goal

This SOP describes the standardized protocols used for Cell free DNA extractions from various body fluids and tissue sources.

Plasma, CSF, ASC, PreiC and PEL cfDNA extraction

Symbol □ denotes sample volumes of 1 ml, symbol Δ denotes sample volumes of 2 ml and symbol ● denotes sample volumes of 3 ml.

1. Pipet □ 100 µl Δ 200 µl, or ● 300 µl QIAGEN Proteinase K into a 15 ml centrifuge tube.
2. Add □ 1 ml, Δ 2 ml, or ● 3 ml of sample to the tube.
3. Add □ 0.8 ml, Δ 1.6 ml, or ● 2.4 ml Buffer ACL (containing 1.0 µg carrier RNA). Close the cap and mix by pulse-vortexing for 30 s.
4. Make sure that a visible vortex forms in the tube. In order to ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.
   Note: Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.
5. Incubate at 60°C for 30 min.
6. Place the tube back on the lab bench and unscrew the cap.
7. Add □ 1.8 ml, Δ 3.6 ml, or ● 5.4 ml Buffer ACB to the lysate in the tube. Close the cap and mix thoroughly by pulse-vortexing for 15–30 s.
8. Incubate the lysate–Buffer ACB mixture in the tube for 5 min on ice.
9. Insert the QIAampMini column into the VacConnector on the QIAvac 24 Plus. Insert a 20 ml tube extender into the open QIAamp Mini column.
10. Make sure that the tube extender is firmly inserted into the QIAamp Mini column in order to avoid leakage of sample.
    Note: Keep the collection tube for the dry spin in step 13.
11. Carefully apply the lysate–Buffer ACB mixture the tube extender of the QIAamp Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.
12. Please note that large sample lysate volumes may need up to 10 minutes to pass through the QIAamp Mini membrane by vacuum force.

Note: To avoid cross-contamination, be careful not to move the tube extenders over neighboring QIAamp Mini Columns.

13. Apply 600 µl Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
14. Apply 750 µl Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
15. Apply 750 µl of ethanol (96–100%) to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of ethanol has been drawn through the spin column, switch off the vacuum pump and release the pressure to 0 mbar.
16. Close the lid of the QIAamp Mini column. Remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube, and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
17. Place the QIAamp Mini Column into a new 2 ml collection tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.
18. Place the QIAamp Mini column in a clean 1.5 ml elution tube and discard the 2 ml collection tube from step 14. Carefully apply 50–70 µl of Buffer AVE to the center of the QIAamp Mini membrane. Close the lid and incubate at room temperature for 3 min.
19. Centrifuge in a microcentrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min to elute the nucleic acids.
20. Optional: For maximum cfDNA yield, repeat elution once as described in steps 18-19.

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Urine cfDNA extraction

Symbol □ denotes sample volumes of 1 ml, symbol Δ denotes sample volumes of 2 ml and symbol ● denotes sample volumes of 3 ml.

1. Pipet □ 125 µl Δ 250 µl, or ● 375 µl QIAGEN Proteinase K into a 50 ml centrifuge tube.
2. Add □ 1 ml, Δ 2 ml, or ● 3 ml of sample to the tube.
3. Add □ 1 ml, Δ 2 ml, or ● 3 ml of Buffer ACL (containing 1.0 µg carrier RNA) to the tube, and □ 250 µl ml, Δ 500µl, or ● 750µl Buffer ATL. Close the cap and mix by pulse-vortexing for 30 s.
4. Make sure that a visible vortex forms in the tube. In order to ensure efficient lysis, it is essential that the sample and Buffer ACL are mixed thoroughly to yield a homogeneous solution.

   Note: Buffer ATL should be added to the lysis mixture as the last component. Make sure a visible vortex forms in the tube. In order to ensure efficient lysis, it is essential that the sample and Buffers ACL and ATL are mixed thoroughly to yield a homogeneous solution.

   A precipitate may form upon mixing of the components. This will redissolve during the lysis incubation and does not affect the yield of nucleic acids.

   Note: Do not interrupt the procedure at this time. Proceed immediately to step 4 to start the lysis incubation.
5. Incubate at 60°C for 30 min.
6. Place the tube back on the lab bench and unscrew the cap.
7. Add □ 3.6 ml, Δ 5.4 ml, or ● 7.2 ml Buffer ACB to the lysate in the tube. Close the cap and mix thoroughly by pulse-vortexing for 15–30 s.
8. Incubate the lysate–Buffer ACB mixture in the tube for 5 min on ice.
9. Insert the QIAampMini column into the VacConnector on the QIAvac 24 Plus. Insert a 20 ml tube extender into the open QIAamp Mini column.
10. Make sure that the tube extender is firmly inserted into the QIAamp Mini column in order to avoid leakage of sample.
11. Carefully apply the lysate–Buffer ACB mixture the tube extender of the QIAamp Mini column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.
12. Please note that large sample lysate volumes may need up to 10 minutes to pass through the QIAamp Mini membrane by vacuum force.

    Note: To avoid cross-contamination, be careful not to move the tube extenders over neighboring QIAamp Mini Columns.

13. Apply 600 µl Buffer ACW1 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW1 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
14. Apply 750 µl Buffer ACW2 to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of Buffer ACW2 has been drawn through the QIAamp Mini column, switch off the vacuum pump and release the pressure to 0 mbar.
15. Apply 750 µl of ethanol (96–100%) to the QIAamp Mini column. Leave the lid of the column open, and switch on the vacuum pump. After all of ethanol has been drawn through the spin column, switch off the vacuum pump and release the pressure to 0 mbar.
16. Close the lid of the QIAamp Mini column. Remove it from the vacuum manifold, and discard the VacConnector. Place the QIAamp Mini column in a clean 2 ml collection tube, and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.
17. Place the QIAamp Mini Column into a new 2 ml collection tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.
18. Place the QIAamp Mini column in a clean 1.5 ml elution tube and discard the 2 ml collection tube from step 14. Carefully apply 50–70 µl of Buffer AVE to the center of the QIAamp Mini membrane. Close the lid and incubate at room temperature for 3 min.
19. Centrifuge in a microcentrifuge at full speed (20,000 x g; 14,000 rpm) for 1 min to elute the nucleic acids.
20. Optional: For maximum cfDNA yield, repeat elution once as described in steps 18-19.

Number of samples	Buffer ACL (ml) ■	▲	●	Carrier RNA in Buffer AVE (µl)
1	0.9	1.8	2.6	5.6
2	1.8	3.5	5.3	11.3
3	2.6	5.3	7.9	16.9
4	3.5	7.0	10.6	22.5
5	4.4	8.8	13.2	28.1
6	5.3	10.6	15.8	33.8
7	6.2	12.3	18.5	39.4
8	7.0	14.1	21.1	45.0
9	7.9	15.8	23.8	50.6
10	8.8	17.6	26.4	56.3
11	9.7	19.4	29.0	61.9
12	10.6	21.1	31.7	67.5
13	11.4	22.9	34.3	73.1
14	12.3	24.6	37.0	78.8
15	13.2	26.4	39.6	84.4
16	14.1	28.2	42.2	90.0
17	15.0	29.9	44.9	95.6
18	15.8	31.7	47.5	101.3
19	16.7	33.4	50.2	106.9
20	17.6	35.2	52.8	112.5
21	18.5	37.0	55.4	118.1
22	19.4	38.7	58.1	123.8
23	20.2	40.5	60.7	129.4
24	21.1	42.2	63.4	135.0

Table 1: Volumes of Buffer ACL and carrier RNA required for processing □ 1 ml, Δ 2 ml, or ● 3 ml of sample.

FF_OCT Cryo-sectioning

1. Samples brought in dry ice for cry sectioning using the cryotome.

2. Use the setting 20µm for block trimming, and 5-10µm to have a section for staining.

3. Two microcentrifuge tubes should be cooled in the cooling chamber to collect tissue sections for DNA and RNA extractions. A minimum of 5 x 20µm to be collected, stored directly at -80°C freezer.

FF_OCT DNA extraction

Important points before starting:

- This protocol uses the kit QIAGEN DNeasy Blood & Tissue (Cat. No. 69506).
- All centrifugation steps are carried out at room temperature (15–25°C) in a microcentrifuge.
- Vortexing should be performed by pulse-vortexing for 5–10 s.
- Buffer ATL and Buffer AL may form precipitates upon storage. If necessary, warm to 56°C
- until the precipitates have fully dissolved.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Preheat a thermomixer, shaking water bath or rocking platform to 56°C for use in step 2.
- When using frozen tissue, equilibrate the sample to room temperature (15–25°C).
- Avoid repeated thawing and freezing of samples, because this will lead to reduced DNA size.

1. Add 180 µl Buffer ATL to the Fresh Frozen tissue. Make sure the tissue is minced or sliced, so the contact with lysis buffer is optimal.
2. Add 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C until the tissue is completely lysed.
3. Vortex occasionally during incubation. Lysis is usually complete in 1–3 h.
4. Optional: If RNA-free genomic DNA is required, add 4 µl RNase A (100 mg/ml), mix by vortexing, and incubate for 2 min at room temperature.
5. Add 200 µl Buffer AL to the sample, and mix thoroughly by vortexing.
6. Then add 200 µl ethanol (96–100%), and mix again thoroughly by vortexing.
7. Pipet the mixture from step 6 (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided).
8. Centrifuge at 6000 x g (8000 rpm) for 1 min. Discard flow-through and collection tube.
9. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1.

10. Centrifuge for 1 min at 6000 x g (8000 rpm). Discard flow-through and collection tube.
11. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2.
12. Centrifuge for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.
13. Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube and pipet 100 µl Buffer AE directly onto the DNeasy membrane.
14. Incubate at room temperature for 1 min, and then centrifuge for 1 min at 6000 x g (8000 rpm) to elute.
15. Optional: For maximum DNA yield, repeat elution once as described in steps 13-14.

FF_OCT RNA extraction

1. Add 750 µl of TRIzol™ Reagent to the Fresh Frozen tissue. Make sure the tissue is minced or sliced, so the contact with TRIzol™ Reagent is optimal.
2. Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
3. Add 200 µl of chloroform per 750 µl of TRIzol™ Reagent used for lysis, then securely cap the tube.
4. Incubate for 10 minutes.
5. Centrifuge the sample for 15 minutes at 12,000 × g at 4°C. The mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase.
6. Transfer 300 µl of the aqueous phase containing the RNA to a new tube.
7. Optional: If starting with little tissue, add LPA to co-precipitate and visualize the RNA pellet.
8. Add 300 µl of isopropanol to the aqueous phase, per 750 µl of TRIzol™ Reagent used for lysis.
9. Incubate for 10 minutes at room temperature.
10. Centrifuge for 10 minutes at 12,000 × g at 4°C. Total RNA precipitate forms a white gel-like pellet at the bottom of the tube.
11. Discard the supernatant using a pipette (be careful not to lose the pellet).
12. Resuspend the pellet in 500 µl of 75% ethanol per 750 µl of TRIzol™ Reagent used for lysis.
13. Vortex the sample briefly then centrifuge for 5 minutes at 7500 × g at 4°C.
14. Repeat step 12 and 13.
15. Discard the supernatant with a pipette, then centrifuge samples for a short 1 min spin. Remove all remaining ethanol with a 20µl tip.
16. Air dry the RNA pellet for 5–10 minutes. IMPORTANT! Do not dry the pellet by vacuum centrifuge. Do not let the RNA pellet over-dry.
17. Resuspend the pellet in 20–50 µL of RNase-free water.
18. Optional: Incubate in a water bath or heat block set at 55–60°C for 10–15 minutes.
19. Proceed to downstream applications, or store the RNA at –70°C.