Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

# UofS-ETL-EDNA-28

# Co-isolation of DNA/RNA/Protein from Cells Using Qiagen AllPrep® DNA/RNA/Protein Mini Kit

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## APPROVAL PAGE

Revisions to an existing SOP, addition of a SOP change form, or preparation of a new SOP must be reviewed, approved, and signed by the following:

Authored By: Yuwei Xie and John P. Giesy Date: 12/22/2018

Supervisor Review By: John P. Giesy Date: 12/22/2018

Reviewed By: _____ Date: _____
(QA Coordinator)

## DEFINITIONS AND ACRONYMS

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|---|---|
| **ETL** | Environmental Toxicology Laboratory (University of Saskatchewan) |
| **DQO** | Data Quality Objective |
| **DHSE** | Department of Health Safety and Environment (University of Saskatchewan) |
| **QA** | Quality Assurance |
| **QAPP** | Quality Assurance Project Plan |
| **SOP** | Standard Operating Procedure |
| **GWF** | Global Water Futures |

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## 1.0 PURPOSE

The primary purpose of this SOP is to parallelly extract, purify and quantify genomic DNA, RNA and protein from cultured cells.

## 2.0 SCOPE AND APPLICATION

This SOP applies to the ETL for co-isolation of DNA/RNA/protein from cultured cells from the Global Water Futures (GWF) program titled "Next generation solutions to ensure healthy water resources for future generations" (eDNA project) as well as other targeted projects from the Department of Fisheries and Oceans (DFO).

## 3.0 SAFETY CONSIDERATIONS

Biosafety training, laboratory training and medical monitoring requirements are described in the Health and Safety Plan for eDNA project of the GWF program. In addition, there are various safety concerns regarding the preparing, storage, and disposal of sample containers at the ETL. If biological spill happens, the appropriate personnel will be immediately notified and the Department of Health, Safety and Environment (DHSE) will be called in order to assess the hazard. DHSE will also be contacted in the case of chemical spills and will be responsible for the disposal of hazardous wastes.

### 3.1 PERSONAL PROTECTIVE EQUIPMENT

Personal protective equipment (PPE), consisting of nitrile gloves will be worn at all times when handling samples.

## 4.0 EQUIPMENT, MATERIALS, AND REAGENTS

- Freezer (-20 $^{o}$C, -80 $^{o}$C)
- Permanent markers
- Chain of custody forms
- Kim wipes
- Absolute ethanol
- Microcentrifuge (16,000 x g)
- 2 mL sterile microcentrifuge tubes
- 1.5 mL sterile microcentrifuge tubes

- Adjustable Pipettes (1000-µl, 200-µl and 10-µl)
- Disposable aerosol filter pipette tips (1000-µl, 200-µl and 10-µl)
- Dry heat block
- Biosafety cabinet
- Nanodrop 1000
- Qia AllPrep DNA/RNA/Protein Mini kit
- QIAshredder spin column
- DNase I stock solution (Dissolve 1500 Kunitz units solid DNase I in 550 µl of the RNase-free water)
- 14.3 M β-mercaptoethanol (β-ME)
- Sterile, RNase-free pipet tips
- Microcentrifuge (with rotor for 2 ml tubes)
- Centrifuge (with rotor for 15 ml tubes, 4000 g RCF available)
- 96–100% ethanol
- 70% ethanol in water
- Disposable gloves
- 5% (w/v) sodium dodecyl sulfate (SDS)
- Qubit V4
- Qubit® DNA HS Assay Kits
- Qubit® Protein Assay Kits
- Qubit® RNA HS Assay Kits
- Qubit™ RNA IQ Assay Kit
- Qubit® assay tubes (Life Technologies, Cat. no. Q32856)
- Spray bottle with Invitrogen™ RNase Away™ Decontamination Reagent
- Spray bottle with 70% ethanol
- Spray bottle with 10% bleach
- Spray bottle with di Water

## 5.0 METHOD, PROCEDURES, AND REQUIREMENTS

The following procedures describe methods for co-isolating DNA/RNA/protein from cultured cells in a stepwise fashion and explain the reasoning behind the techniques. Detailed below are the preparatory procedures, molecular techniques, and procedures for sample documentation and preservation.

### 5.1 Mobilization and Training

This section describes requirements for mobilization for the necessary lab-work, as well as the necessary safety training. The objective of this section is to ensure that all of the necessary preparatory work has been conducted to enable the successful completion of

the overall project. Mobilization for the necessary lab-work entails maintenance and decontamination of equipment and biosafety training in accordance with the site Health and Safety Plan (HASP).

5.2 **Decontamination of instruments and laboratory areas before and after isolation**

A. Wipe down surfaces of all points of contact, including bench tops, pipettes, and fridge/freezer handles, using 10% bleach. Let bleach remain on surfaces for 15 minutes.
B. After 15 minutes, use a DI water-dampened paper towel to remove bleach residue.
C. Use a 70% alcohol dampened paper towel to help quickly dry the surfaces. **Note**: **For PCR PRE-station, DO NOT use buffers containing ethanol to clean clear, acrylic surface.**
D. If necessary, repeat the cleaning procedure from step B.
E. For the biosafety cabinet and PCR PRE-station, turn on the UV-light for at least 15 min.
F. Decontaminate laboratory equipment during work using RNase AWAY™ Surface Decontaminant (Thermo Scientific™)
G. Wipe down surfaces of bench tops, pipettes, and racks using RNase AWAY™ Surface Decontaminant, then wipe dry with dust-free paper or rinse clean.

**5.3 Co-isolation of DNA/RNA/Protein from cultured cells Using Qia AllPrep® DNA/RNA/Protein Mini Kit**

**Notes before starting**

- β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. Add 10 µl β-ME per 1 ml Buffer RLT. Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature (15–25°C) for up to 1 month. Label the date.
- Buffer RPE, Buffer AW1, and Buffer AW2 are each supplied as a concentrate. Before using for the first time, add the appropriate volume of ethanol (96–100%) as indicated on the bottle to obtain a working solution.
- Buffer RLT may form a precipitate upon storage. If necessary, redissolve by warming, and then place at room temperature.
- Preheat Buffer EB to 70°C to ensure optimal DNA elution.
- Pre-chill Qiagen TissueLyser Adaptors at -20°C
- Preheat dry heat block at 95°C

**Note: At least one negative control should be conducted during isolation.**

### *5.3.1 Sample homogenization*

1) Determine the number of cells (do not use more than 1 x 107 cells).
   (A) Pellet the appropriate number of cells by centrifuging for 5 min at 300 x g in a 15-mL or 50-mL falcon tube. Carefully remove all supernatant by aspiration, and proceed to step 2).
   Or (B) Trypsinize, collect and pellet cells grown in a monolayer in cell-culture vessels or flasks. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, and add 0.10–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free polypropylene centrifuge tube, and centrifuge at 300 x g for 5 min. Completely aspirate the supernatant, and proceed to step 2.
   **Note: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, reducing the nucleic acid yields and purity conditions for DNA/RNA purification.**
2) In the tube with pelleted cells, add 600 µl Buffer RLT and suspend the pellet cells by pipetting. **Note: β-ME must be added to Buffer RLT before use**.
3) Pipet the lysate directly into a QIAshredder spin column placed in a 2 ml collection tube, and centrifuge for 2 min at full speed.
4) Transfer the homogenized lysate to an AllPrep **DNA** spin column placed in a 2 ml collection tube. Close the lid gently, and centrifuge for 30 s at 16,000 x g. **Transfer the flow through into a new 1.5-mL tube for RNA purification in steps 6) – 16)**.
5) Place the AllPrep DNA spin column in a new 2 ml collection tube (supplied), and store at room temperature (15–25 °C) for later **DNA purification in steps 24) – 27)**. Use the flow-through for RNA purification. **Note: Do not store the AllPrep DNA spin column at room temperature for long periods. Do not freeze the column.**

### *5.3.2 Purification of total RNA*

6) To the flow-through from **steps 4)**, add 400 µl absolute ethanol. Mix well by pipetting. Do not centrifuge. Proceed immediately to **step 7)**.
7) Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube (supplied). Close the lid gently, and centrifuge for 15 s at 16,000 x g. **Transfer the flow-through to a 2 ml tube (supplied) for protein purification in steps 17) – 23)**.
   Note: Reuse the collection tube during RNA purification. If the sample volume exceeds 700 µl, centrifuge successive aliquots in the same RNeasy spin column. **Transfer the flow-through after each centrifugation to the 2 ml tube for purification of protein**.
8) Add 350 µl Buffer RW1 to the RNeasy spin column, and centrifuge for 15 s at 16,000 x g to wash the spin column membrane. Discard the flow through.
9) Add 10 µl DNase I stock solution to 70 µl Buffer RDD. Mix by gently inverting the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.

10) Add the DNase I incubation mix (80 µl) directly to the RNeasy spin column membrane, and place on the benchtop (20–30 °C) for 15 min.
11) Add 500 µl Buffer RW1 to the RNeasy spin column, and centrifuge for 15 s at 16,000 x g. Discard the flow-through.
12) Repeat step 11) once.
13) Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently, and centrifuge for 15 s at 16,000 x g to wash the spin column membrane. Discard the flow-through.
14) Repeat **step 13)** once.
15) Centrifuge the column for 2 min at 16,000 x g to dry the column.
16) Place the RNeasy spin column in a new 1.5 ml collection tube (supplied). Add 50 µl RNase-free water directly to the spin column membrane. Close the lid gently, and centrifuge for 1 min at 16,000 x g to elute the RNA. **Store purified RNA at –80 °C after QC.**

### *5.3.3 Total protein precipitation*

17) Add 1 volume of Buffer APP to the flow-through from **step 7**. Mix vigorously and incubate at room temperature for 10 min to precipitate protein.
18) Centrifuge at 16,000 x g for 10 min, and carefully decant the supernatant.
19) Add 500 µl of 70% ethanol to the protein pellet. Centrifuge at full speed for 1 min, and remove the supernatant by using a pipet or by decanting as much liquid as possible.
20) Dry the protein pellet for 10 min at room temperature.
**Note: Genomic DNA purification can be proceeded during steps 17-20.**
21) Add up to 100 µl 5% SDS solution and mix vigorously to dissolve the protein pellet.
22) Incubate for 5 min at 95°C to completely dissolve and denature protein. Then cool the sample to room temperature.
23) Centrifuge for 1 min at full speed to pellet any residual insoluble material. The dissolved protein can be stored at –80 °C.

### *5.3.4 Purification of DNA*

24) Add 500 µl Buffer AW1 to the AllPrep DNA spin column from **step 5)**. Close the lid gently, and centrifuge for 15 s at 16,000 x g. Discard the flow through.
25) Add 500 µl Buffer AW2 to the AllPrep DNA spin column. Close the lid gently, and centrifuge for 15 s at 16,000 x g to wash the spin column membrane.
26) Centrifuge the column for 2 min at 16,000 x g to dry the column.
27) Place the AllPrep DNA spin column in a new 1.5 ml collection tube (supplied). Add 100 µl Buffer EB (preheated to 70 °C) directly to the spin column membrane and close the lid. Incubate at room temperature (15–25 °C) for 2 min, and then centrifuge for 1 min at 16,000 x g to elute the DNA. **Store purified DNA at –80 °C after QC.**

## 5.4 Quality control of isolated RNA

### *5.4.1 Purity check and quantify the isolated RNA using NanoDrop 1000*

28) Turn on NanoDrop computer and open ND-1000 software.
29) Wipe NanoDrop pedestal with a clean Kimwipe.
30) In NanoDrop software, select Nucleic Acid, RNA-40 mode.
31) Pipette 1.5 µl of sterile DNase-free water onto the pedestal of NanoDrop. Press OK. After calibrating, wipe NanoDrop pedestal with a clean Kimwipe.
32) Load 1.5 µl of elution buffer onto the pedestal of NanoDrop. Select "Blank". Waiting for Blank to be read (about 10 s).
33) Wipe NanoDrop pedestal with a clean Kimwipe.
34) Enter first sample and name. Apply 1.5 µl of RNA sample onto the pedestal and select "Measure".
35) Save file to an appropriate folder on the computer and print report.
36) Reapply 1.5 µl of sterile DNase-free water onto the pedestal and wipe pedestal with a clean Kimwipe.
37) Close NanoDrop.

### *5.4.2 Quantify the RNA with low concentration (< 10 ng/µl) with Qubit® RNA HS Assay Kits*

38) Set up the required number of 0.5-mL tubes for standards and samples. The Qubit® RNA HS Assay requires 2 standards.
39) Label the tube lids.
40) Prepare the Qubit® working solution by diluting the Qubit® RNA HS Reagent 1:200 in Qubit® RNA HS Buffer. Use a clean plastic tube each time you prepare Qubit® working solution. Note: Do not mix the working solution in a glass container.
41) Add 190 µl of Qubit® working solution to each of the tubes used for standards.
42) Add 10 µl of each Qubit® standard to the appropriate tube, then mix by vortexing 2–3 seconds. Be careful not to create bubbles.
43) Add 198 µl Qubit® working solution to individual assay tubes.
44) Add 2 µl of each RNA sample to the assay tubes containing the correct volume of Qubit® working solution, then mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µl.
45) Allow all tubes to incubate at room temperature for 2 minutes.
46) Measure the concentration of RNA samples using Qubit® 4.0 Fluorometer.
47) Transfer data file to an appropriate folder on the computer and print report.

### *5.4.3 Check the degradation of RNA sample using the Qubit™ RNA IQ Assay Kit*

**Note: The quality of total RNA (RIN) also can be checked by Agilent Bioanalyzer 2100 with RNA kit.**

48) Set up the required number of 0.5-mL tubes for standards and samples. The Qubit® RNA IQ Assay requires 3 standards.

49) Label the tube lids.
50) Prepare the Qubit® working solution by diluting the Qubit® RNA IQ Reagent 1:200 in Qubit® RNA IQ Buffer. Use a clean plastic tube each time you prepare Qubit® working solution. Note: Do not mix the working solution in a glass container.
51) Add 190 µl of Qubit® working solution to each of the tubes used for standards.
52) Add 10 µl of each Qubit® standard to the appropriate tube, then mix by vortexing 2–3 seconds. Be careful not to create bubbles.
53) Add 198 µl Qubit® working solution to individual assay tubes.
54) Add 2 µl of each RNA sample to the assay tubes containing the correct volume of Qubit® working solution, then mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µl.
55) Allow all tubes to incubate at room temperature for 2 minutes.
56) Measure the concentration of RNA samples using Qubit® 4.0 Fluorometer.

**5.5 Quality control of isolated Protein**

### *5.5.1 Purity check and quantify the isolated protein using NanoDrop 1000*

57) Turn on NanoDrop computer and open ND-1000 software.
58) Wipe NanoDrop pedestal with a clean Kimwipe.
59) In NanoDrop software, select Protein A280.
60) Pipette 2 µl of sterile DNase-free water onto the pedestal of NanoDrop. Press OK. After calibrating, wipe NanoDrop pedestal with a clean Kimwipe.
61) Load 2 µl of 5% SDS buffer onto the pedestal of NanoDrop. Select "Blank". Waiting for Blank to be read (about 10 s).
62) Wipe NanoDrop pedestal with a clean Kimwipe.
63) Enter first sample and name. Apply 2 µl of protein sample onto the pedestal and select "Measure".
64) Save file to an appropriate folder on the computer and print report.
65) Reapply 1.5 µl of sterile DNase-free water onto the pedestal and wipe pedestal with a clean Kimwipe.
66) Close NanoDrop.

### *5.5.2 Quantify the protein with Qubit® Protein Assay Kits*

67) Dilute the protein (2 µl in 8 µl di$H_2O$)
68) Set up the required number of 0.5-mL tubes for standards and samples. The Qubit® Protein Assay requires 3 standards.
69) Label the tube lids.
70) In a clean plastic tube, prepare the Qubit® working solution by diluting the Qubit® Protein Reagent 1:200 in Qubit® Protein Buffer.
71) Add 190 µL of Qubit® working solution to each of the tubes used for standards.
72) Add 10 µL of each Qubit® standard to the appropriate tube, then mix by vortexing 2–3 seconds. Be careful not to create bubbles.

73) Add 198 µL Qubit® working solution to individual assay tubes.
74) Add 2 µL each sample to the assay tubes containing the correct volume of Qubit® working solution, then mix by vortexing 2–3 seconds.
75) Allow all tubes to incubate at room temperature for 15 minutes.
76) Measure the concentration of protein samples using Qubit® 4.0 Fluorometer.
77) Quintuple the Qubit results to calculate the concentration of eProtein of each sample.

### 5.6 Quality control of isolated DNA

#### *5.6.1 Purity check and quantify the isolated DNA using NanoDrop 1000*

78) Turn on NanoDrop computer and open ND-1000 software.
79) Wipe NanoDrop pedestal with a clean Kimwipe.
80) In NanoDrop software, select Nucleic Acid.
81) Pipette 1.5 µl of sterile DNase-free water onto the pedestal of NanoDrop. Press OK. After calibrating, wipe NanoDrop pedestal with a clean Kimwipe.
82) Load 1.5 µl of elution buffer onto the pedestal of NanoDrop. Select "Blank". Waiting for Blank to be read (about 10 s).
83) Wipe NanoDrop pedestal with a clean Kimwipe.
84) Enter first sample and name. Apply 1.5 µl of DNA sample onto the pedestal and select "Measure".
85) Save file to an appropriate folder on the computer and print report.
86) Reapply 1.5 µl of sterile DNase-free water onto the pedestal and wipe pedestal with a clean Kimwipe.
87) Close NanoDrop.

#### *5.6.2 Quantify the DNA with low concentration (< 10 ng/µl) with Qubit® dsDNA HS Assay Kits*

88) Set up the required number of 0.5-mL tubes for standards and samples. The Qubit® dsDNA HS Assay requires 2 standards.
89) Label the tube lids.
90) Prepare the Qubit® working solution by diluting the Qubit® dsDNA HS Reagent 1:200 in Qubit® dsDNA HS Buffer. Use a clean plastic tube each time you prepare Qubit® working solution. Note: Do not mix the working solution in a glass container.
91) Add 190 µl of Qubit® working solution to each of the tubes used for standards.
92) Add 10 µl of each Qubit® standard to the appropriate tube, then mix by vortexing 2–3 seconds. Be careful not to create bubbles.
93) Add 198 µl Qubit® working solution to individual assay tubes.
94) Add 2 µl of each eDNA sample to the assay tubes containing the correct volume of Qubit® working solution, then mix by vortexing 2–3 seconds. The final volume in each tube should be 200 µl.
95) Allow all tubes to incubate at room temperature for 2 minutes.
96) Measure the concentration of eDNA samples using Qubit® 4.0 Fluorometer.

97) Transfer data file to an appropriate folder on the computer and print report.

## 6.0 RECORDS, DOCUMENTATION, AND QC REQUIREMENTS

Preserve isolated DNA/RNA/protein sample under -80 $^{o}$C for long-term storage. Sample management will also follow a scripted procedure (ETL-SOP-EDNA-09: Management of eDNA sample: Receiving, Preservation, Storage, Documentation, Decontamination, and Disposal) to ensure samples will not be lost or destroyed. The results of quality control of eDNA samples should be archived properly (hard-copy and electric copy).

## 7.0 RESPONSIBILITIES

**Project Director** — Will oversee and approve all project activities.

**Project Manager** — Will oversee and approve all project activities; review QA reports; approve final project QA needs; authorize necessary actions and adjustments to accomplish program QA objectives; and act as liaison between agencies, field staff.

**Quality Assurance (QA) Manager** — Will oversee all QA activities to ensure compliance with contract specifications; initiate audits on work completed by project personnel and subcontractors, including analytical laboratories and independent data validation contractors; review program QA activities, quality problems, and quality-related requests. In response to field and analytical findings, this person will approve the corrective actions. This person will report quality non-conformances to the Project Manager and review all pertinent portions of the deliverables before they are transmitted to ensure conformance with QA/QC procedures and quality work product.

**Data Manager** — Will oversee data management for this project. This person is responsible for the structure, organization, format, implementation, and operation of the project database.

**Field Team Leader** — Will oversee field activities and supervise the field crews. This person will ensure that proper sample collection, preservation, storage, transport, and COC QC procedures are followed. This person will inform the Project QA Manager when field problems occur, and will communicate and document corrective actions taken. The Field Team Leader will discuss field activities with the Project Manager.

**Laboratory Project Manager** — The laboratory project manager for this project is the person responsible for assuring that the analysis of all samples is performed in accordance with the QAPP and the laboratory's quality assurance manual. In addition,

the Laboratory Project Manager performs the final laboratory review of project data packages for completeness and compliance with project requirements.

## 8.0 REFERENCES

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MO BIO. PowerWater® RNA Isolation Kit. Version: 05302013.
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Qiagen. TissueLyser Handbook. Ocectmober 2010, 2nd edition.
Thermo Scientific. NanoDrop 1000 Spectrophotometer User's Manual. Rev. V3.8
Thermo Scientific. Qubit™ 4 Fluorometer manual. Pub. No. MAN0017209. Rev. B.0.
Thermo Scientific. Qubit® RNA HS Assay Kits manual. Pub. No. MAN0002327. Rev. A.0.
Thermo Scientific. Qubit® RNA IQ Assay Kits manual. Pub. No. MAN0017405. Rev. B.0.
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