Interreg Italia-Österreich
precanmed

UNIUD-CCO-SOP-3.0

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DNA, RNA & PROTEIN EXTRACTION FROM ORGANOIDS

Purpose

The purpose of SOP 3.0 is to describe how to extract DNA, RNA and protein from intestinal organoids.

Scope

SOP 3.0 is intended to cover all resources, personnel and equipment in the intestinal organoids laboratory.

Introduction

Collaborators at UniUD prepare intestinal organoids. The preparation of biomolecules such as protein, RNA and DNA is central for the molecular characterization of organoid cultures.

1. Reagents

Reagent	Catalogue number and company
PBS without Ca2+, Mg2+ (PBS)	#BE17-512F Lonza
BD Cell Recovery Solution	#354253 Corning
Advanced DMEM/F12	#12634028 Life Technologies
Penicillin-Streptomycin (P/S)	#15070063 Life Technologies
GlutaMAX™ Supplement (Glutamax)	#35050038 Life Technologies
HEPES	#15630056 Life Technologies
QIAamp DNA Blood Mini Kit	#51104 Qiagen
TRIzol reagent	#15596018 Invitrogen
NaCl	#S7653 Sigma-Aldrich

Trizma	#T1503 Sigma-Aldrich
Triton-X100	#T8787Sigma-Aldrich
EDTA	#E5134 Sigma Aldrich
protease inhibitor cocktail	#P8340 Sigma Aldrich
DTT	#10197777001 Sigma Aldrich
PMSF	#P7626 Sigma Aldrich
NaF	#S6521 Sigma Aldrich
Na3VO4	#S6508 Sigma Aldrich
Bradford protein assay reagent	#5000006 Biorad

2. Equipment

- Pipetaid (SN603280198 Euroclone)
- Centrifuge (5810R Eppendorf, Sorvall Legend Micro21R ThermoScientific)
- Nanodrop (Nanodrp 2000 ThermoScientific)

3. Plastics

- 24-multiwell TC-Plate (#GR662160 Greiner Bio-One)
- Pipettes (#EPS05N, #EPS10N, #EPS25N Euroclone)
- 10 µl, 200 µl and 1000 µl pipette tips (#ECTD50010RN, #ECTD50200RN, #ECTD51000RN Euroclone)
- 1.5 ml microcentrifuge tubes (#ET3415 Euroclone)
- 2 ml cryogenic vials (#ECC3212RB Euroclone)
- 100 mm petri dish (#ET2100 Euroclone)
- 50 ml and 15 ml conical tubes (#ET5050B, #ET5015B Euroclone)
- 100 µm cell strainer (#ET6100 Euroclone)

5. Solutions

GF:

- 500 ml Advance DMEM/F12
- 5 m of P/S 100X
- 5 ml of HEPES 1M
- 5 ml of Glutamax 100X

Lysis Buffer:

- 50mM Tris-HCl pH 7.4
- 150mM NaCl
- 1mM EDTA
- 1% w/v Triton X-100)
- 1mM protease inhibitor cocktail
- 1mM DTT
- 0.5mM PMSF
- 1mM NaF
- 1mM Na3VO4

6. Procedure

6.1 DNA extraction

Extraction of DNA was performed using QIAamp DNA Blood Mini Kit Qiagen (Cat No./ID: 51104).

6.1.1 Sample preparation

Perform all procedures on ice unless indicated otherwise. Usually DNA was. extracted from 12 wells of organoid cultures (grown in 24 well plate).

1. Remove medium and add 1 ml of cold GF- and make Matrigel loose with p1000 tip. Pipet up and down. Recover suspension in a 15 ml canonical tube. Repeat procedure.
2. Centrifuge at 800 rpm for 3 min at 4˚C. Discard supernatant.
3. Incubate organoids with BD Cell Recovery Solution for 23-30 min on ice.
4. Centrifuge at 800 rpm for 5 min at 4˚C. Discard supernatant.
5. Wash pellet with PBS. Centrifuge at 800 rpm for 5 min at 4˚C. Discard supernatant.
6. Proceed with QIAamp DNA Blood Mini Kit according to the manu-

facturer's instructions (elute DNA in 50 µl of distilled water).

7. Determinate the DNA concentration with Nanodrop.
8. Store samples at -20°C.

See ***Figure 1.***

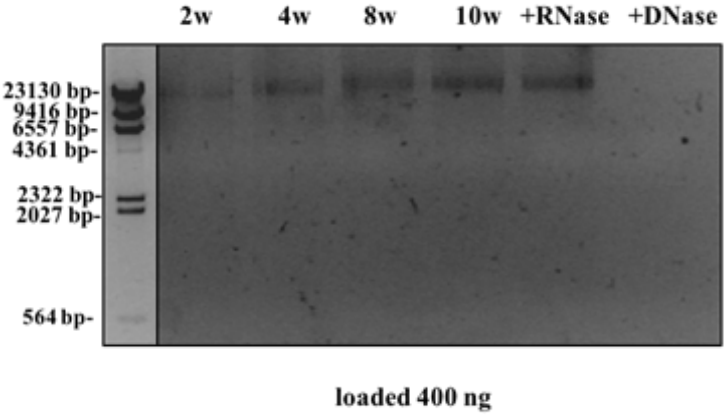

Figure 1. Agarose Gel Electrophoresis of DNA extracted form tumor organoids. An agarose gel electrophoresis was performed with 400 ng of genomic DNA. Two aliquots of DNA were treated with RNase and DNase. w= number of wells of cultured organoids used for DNA preparation.

6.2 RNA extraction

Extraction of RNA was performed using Trizol reagent.

6.2.1 Sample preparation

Perform all procedures on ice unless indicated otherwise. Usually extract RNA from 4 wells.

1. Remove medium and add 1 ml of cold GF- and make Matrigel loose with p1000 tip. Pipet up and down. Recover suspension in a 15 ml canonical tube. Repeat procedure.
2. Centrifuge at 800 rpm for 5 min at 4°C. Discard supernatant.
3. Incubate organoids with BD Cell Recovery Solution for 23-30 min on ice.
4. Centrifuge at 800 rpm for 5 min at 4°C. Discard supernatant.
5. Wash pellet with PBS. Centrifuge at 800 rpm for 5 min at 4°C. Discard supernatant.
6. Proceed with TRIzol protocol (#15596018 Invitrogen) according to the manufacturer's instructions (elute DNA in 20 µl of RNase-free water).

7. Determine the RNA yield using Nanodrop methods.

8. Store samples at -80°C.

See ***Figure 2***

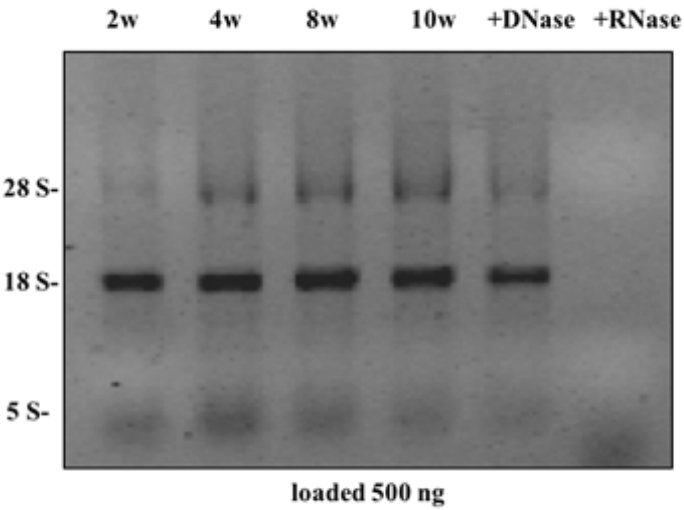

Figure 2. Agarose Gel Electrophoresis of RNA extracted form tumor organoids. An agarose gel electrophoresis was performed with 500 ng of total RNA. Two aliquots of RNA were treated with DNase and RNase. w= number of wells of cultured organoids used for DNA preparation.

6.3 Protein extraction

Extraction of RNA was performed using Trizol reagent.

6.3.1 Sample preparation

Perform all procedures on ice unless indicated otherwise. Usually extract protein from 4 wells.

1. Remove medium. and add 1 ml of cold GF- and make Matrigel loose with p1000 tip. Pipet up and down. Recover suspension in a 15 ml canonical tube. Repeat procedure.
2. Centrifuge at 800 rpm for 5 min at 4°C. Discard supernatant.
3. Incubate organoids with BD Cell Recovery Solution for 23-30 min on ice.
4. Centrifuge at 800 rpm for 5 min at 4°C. Discard supernatant.

5. Wash pellet with PBS. Centrifuge at 800 rpm for 5 min at 4°C. Discard supernatant.
6. Resuspend pellet in lysis buffer (50mM Tris-HCl pH 7.4, 150mM NaCl, 1mM EDTA, 1% w/v Triton X-100) supplemented with 1mM protease inhibitor cocktail, 1mM DTT, 0.5mM PMSF, 1mM NaF and 1mM Na3VO4 for 30 min at 4 °C.
7. Centrifugation at 15,000 x g for 20 min at 4 °C.
8. Collect the supernatant.
9. Determine the protein concentration using Bio-Rad protein assay reagent (#5000006 Biorad) according to the manufacturer's instructions.

See Figure 3

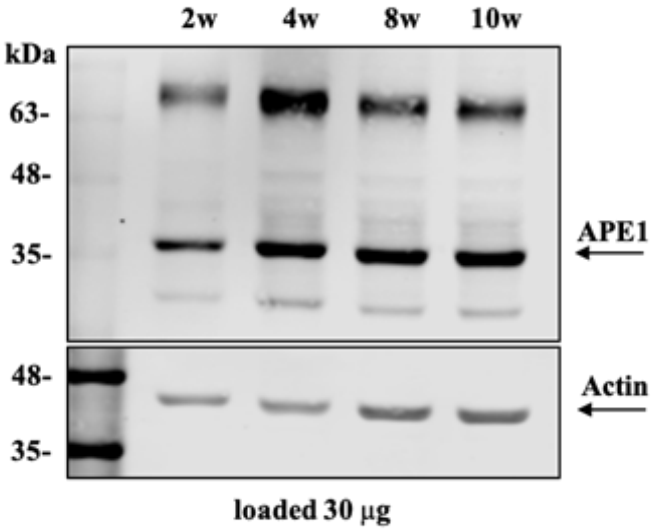

Figure 3. Western blot analysis of proteins extracted form tumor organoids. A total of 30 µg of extracted proteins were loaded. Western blot was performed with an anti-Ape1 antibody. Actin was used as loading control. w= number of wells of cultured organoids used for DNA preparation.