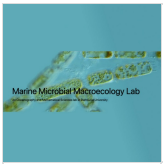

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Total RNA and DNA in microalgae: The extraction and quantification

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1 *Works for me* This protocol is published without a DOI.

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ABSTRACT

This assay protocol for total RNA and DNA is adapted from Berdalet E. et al. and Liefer J.D. et al.

RNA and DNA are extracted from microalgae samples and then quantified by fluorochrome SYBR Green II.

Berdalet E, Roldán C, Olivar MP, Lysnes K. Quantifying RNA and DNA in planktonic organisms with SYBR Green II and nucleases. Part A. Optimisation of the assay. Scientia Marina. https://doi.org/10.3989/scimar.2005.69n11

Berdalet E, Roldán C, Olivar MP. Quantifying RNA and DNA in planktonic organisms with SYBR Green II and nucleases. Part B. Quantification in natural samples. Scientia Marina. https://doi.org/10.3989/scimar.2005.69n117

Liefer JD, Garg A, Fyfe AH, Irwin AJ, Benner I, Brown CM, Follows MJ, Omta AW and Finkel ZV. The Macromolecular Basis of Phytoplankton C:N:P Under Nitrogen Starvation. Front. Microbiol.. https://doi.org/10.3389/fmicb.2019.00763

PROTOCOL CITATION

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KEYWORDS

RNA, DNA, SYBR Green II, DNase, RNase, microalgae, fluorescence

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GUIDELINES

The requirement of micralgae sample is of no higher than 50 ug protein (or 100 ug carbon) per sample, considering the optimized linear range of standard curve for DNA is 0~100 ng/mL and RNA is 0~300 ng/mL.

Microalgae samples are collected on polycarbonate filter and kept frozen under -80 ºC.

MATERIALS

NAME	CATALOG #	VENDOR
Nuclease decontamination solution	11-05-03-01	IDT
SYBR™ Green II RNA Gel Stain (10000X concentrate in DMSO stored at -20 C)	S7564	Thermofisher
Tris solution (1M pH 8 RNase-free)	AM9851	Thermofisher
EDTA buffer (0.5M DNase/RNase free)	EDT333.100	Bioshop
N-Lauroylsarosine sodium salt solution (20% RNase/DNase free)	L744-50mL	Sigma Aldrich
DEOXYRIBONUCLEASE1 RNase and Protease Free	DRB002.10	Bioshop
Ribonuclease A from bovine pancreas	R6513-50MG	Sigma Aldrich
Deoxyribonucleic acid from calf thymus	D4522-1MG	Sigma Aldrich
E. coli Total RNA	AM7940	Thermofisher
Magnesium chloride solution	63069-100ML	Sigma Aldrich
Calcium chloride solution	21115-100ML	Sigma Aldrich
UltraPure™ DNase/RNase-Free Distilled Water	10977023	Thermofisher

MATERIALS TEXT

FreeZone® 2.5 L Benchtop Freeze Dryers

Labconco® 700202000

A2 Biosafety Cabinet

4' REDISHIP Purifier Logic+

Labconco 302419000

BT Barrier Pipet Tips

Pre-Sterile

Neptune® BT1250, BT100, BT10

Filter forceps
blunt end, stainless steel
Millipore XX6200006P

LYSING TUBES
MATRIX D 2 mL/15 mL
MP BIOMEDICALS 116913500/116933050

Microcentrifuge Tubes
1.7 mL/0.6 mL
Axygen Scientific MCT-175-C/MCT-060-L-C

Screw-Cap Centrifuge Tube
5 mL
VWR 10002-738

Falcon® Centrifuge Tubes
Polypropylene, Sterile, 15 mL
Corning® 352096

Falcon® Centrifuge Tubes
Polypropylene, Sterile, 50 mL
Corning® 352070

Fastprep-24 5G™ Sample Preparation Instrument
MP Biomedicals 116005500

CENTRIFUGE 5430 R

Eppendorf MP2231000510

VWR ANALOG VORTEX MIXER

VWR 10153-838
With tube insert

SHAKING INCUBATOR
71L

Corning® LSE™ 6753

Finnpipette Stepper Pipette

Thermo Scientific™ 4540000

Finntip™ Stepper Pipette Tips
500 uL

Thermo Scientific™ 9404170

96-Well Black Microplates
Polystyrene

Greiner Bio-One 655076

Black Vinyl Films for Fluorescence and Photoprotection

VWR 89087-692

Varioskan LUX Multimode Microplate Reader

Thermo Fisher VL0L00D0

EQUIPMENT

NAME	CATALOG #	VENDOR
BT Barrier Pipet Tips	BT1250, BT100, BT10	VWR international Ltd
Falcon® Centrifuge Tubes	352096	VWR international Ltd
A2 Biosafety Cabinet	302419000	
CENTRIFUGE 5430 R	MP2231000510	
VWR ANALOG VORTEX MIXER	10153-838	VWR international Ltd
Finnpipette Stepper Pipette	4540000	Fisher Scientific
Microcentrifuge Tubes	MCT-175-C/MCT-060-L-C	VWR international Ltd
Screw-Cap Centrifuge Tube	10002-738	VWR international Ltd
Falcon® Centrifuge Tubes	352070	VWR international Ltd
SHAKING INCUBATOR	6753	
Varioskan LUX Multimode Microplate Reader	VL0L00D0	
Filter forceps	XX6200006P	Emdmillipore
LYSING TUBES	116913500/116933050	VWR international Ltd
Fastprep-24 5G™ Sample Preparation Instrument	116005500	VWR international Ltd
FreeZone® 2.5 L Benchtop Freeze Dryers	700202000	VWR international Ltd
Finntip™ Stepper Pipette Tips	9404170	Fisher Scientific
96-Well Black Microplates	655076	
Black Vinyl Films for Fluorescence and Photoprotection	89087-692	

RNA and DNA extraction 2h

1 Freeze dry samples.

Freeze-drying should be as short as possible to reduce sample degradation.

2 Expose biosafety cabinet to UV light for **00:15:00** and clean working surface with decontamination solution.

3 Prepare falcon tubes and tube rack in biosafety cabinet

Number	Tube type	Usage
2	5 mL falcon tubes	EDTA20% sarcosine
3	15 mL falcon tubes	1 M Tris, 5 mM Tris, 1% STEB

4 Prepare Tris buffer [M]**5 mM** pH**8.0**

4.1 Pour [M]**1 M** pH**8.0** Tris into an RNase free 15 mL Falcon tube

4.2 Add **2.5 mL** **1 M** pH **8.0** Tris into 500 mL RNase free water in the original package.

5 Prepare STEB (**1 %**)

Require 500 uL for each sample and 500 uL for preparing 0.05% STEB in assay.

5.1 Pour sarcosine (**20 %**) into an RNase free 5 mL falcon tube.

5.2 Pour EDTA (**0.5 M**) into an RNase free 5 mL falcon tube.

5.3 Pour Tris buffer (**5 mM** , pH **8.0**) into an RNase free 15 mL falcon tube.

5.4 Mix **500 µl** sarcosine (**20 %**) , **10 µl** EDTA (**0.5 M**) and **9 mL** + **490 µl** Tris buffer (**5 mM** , pH **8.0**) to obtain STEB (**1 %**).

6 Prepare ice bath

7 Remove samples from -80°C freezer and place **On ice** .

8 Add **500 µl** Tris buffer (**5 mM** , pH **8.0**) and **500 µl** STEB (**1 %**) to the bead tube. Place tubes **On ice** .

9 Transfer sample/blank filter to the tube.
Forcepts must be cleaned by **70 % volume** ethanol.
Blank filter should be the same type as the one with samples collected.

10 Vortex immediately then put back **On ice** . 20s

11 Disrupt samples on the bead mill at 6.5 m/s. 30s

12 Keep tubes **On ice** . Check the label on each tube, restore the label if it fades. 1m 30s

13 Disrupt samples on the bead mill at 6.5 m/s. 30s

14 Keep tubes **On ice** . Check the label on each tube, restore the label if it fades. 1m 30s

15 Disrupt samples on the bead mill at 6.5 m/s 30s

16 Keep tubes **On ice** . Check the label on each tube, restore the label if it fades. 1m 30s

17 Disrupt samples on the bead mill at 6.5 m/s. 30s

18 Continuously shake homogenate in a multi-head vortex at the highest reasonable speed for **01:00:00** 1h

Room temperature

19 In the biosafety cabinet, aliquot 150 uL homogenates into RNase free 600 uL tubes.

20 Store at **-80 °C** .

Prepare standard and nucleases

21 RNA primary standard ([M] **200 ug/ml**)

21.1 Add **1 mL** Tris buffer ([M] **5 mM** , pH **8.0**) to the original package and vortex.

21.2 Aliquot 30 uL by stepper with sterile tip to 600 uL RNase free microtubes. Keep frozen at **-80 °C** .

22 DNA primary standard ([M] **500 ug/ml**)

22.1 Add **2 mL** Tris buffer ([M] **5 mM** , pH **8.0**) to the original package and vortex.

22.2 Aliquot 10 uL by stepper with sterile tip to 600 uL RNase free microtubes. Keep frozen at **-80 °C** .

23 RNase primary stock ([M] **10 mg/ml**)

23.1 Add **5 mL** Tris buffer ([M] **5 mM** , pH **8.0**) to the original package and vortex.

23.2 Aliquot 30 uL by stepper with sterile tip to 600 uL RNase free microtubes. Keep frozen at **-20 °C** .

24 DNase primary stock ([M] **5 mg/ml** = 10,000 U/mL)

24.1 Add **1 mL** Tris buffer ([M] **5 mM** , pH **8.0**) to the original package and vortex.

24.2 Aliquot 100 uL by stepper with sterile tip to 600 uL RNase free microtubes. Keep frozen at **-20 °C** .

The assay with nucleases

25 Prepare ice bath.

26 Expose biosafety cabinet to UV light for **00:15:00** and clean working surface with decontamination solution.

27 Prepare falcon tubes, microtubes and tube racks in biosafety cabinet

* Maximum number of samples (including blanks) per assay is 20.

Number	Tube type	Usage
6	5 mL falcon tubes	1 M $MgCl_2$ 1 M $CaCl_2$ WS-A WS-B WS-C SG-II WS
2	15 mL falcon tubes	5 mM Tris, 0.05% STEB
3	600 uL RNase free tubes	RNase 900 mM $MgCl_2$ 900 mM $CaCl_2$
2	1.7 mL RNase free tubes	RNA standard DNA standard
33	1.7 mL RNase free tubes	standards reaction
N=Samples + blanks	1.7 mL RNase free tubes	sample dilution
3N	1.7 mL RNase free tubes	diluted sample reaction
5	Microtube racks	Microtubes
1	Tube rack	Falcon tubes

28 Organize and label the tubes as shown below

Set 1: Sample dilution

1	2	3	4	5	6	7	8	9	…	Blank

Set 2: Standards

Tubes A	R1A	R2A	R3A	R4A	R5A	R6A	D1A	D2A	D3A	D4A	D5A
Tubes B	R1B	R2B	R3B	R4B	R5B	R6B	D1B	D2B	D3B	D4B	D5B
Tubes C	R1C	R2C	R3C	R4C	R5C	R6C	D1C	D2C	D3C	D4C	D5C

Set 3: Sample reaction

Tubes A	1A	2A	3A	4A	5A	6A	7A	8A	9A	...	BlankA
Tubes B	1B	2B	3B	4B	5B	6B	7B	8B	9B	...	BlankB
Tubes C	1C	2C	3C	4C	5C	6C	7C	8C	9C	...	BlankC

29 Label tubes for reagents as following.

Add Tris buffer ([M] **5 mM** , pH **8.0**) to the reagent tubes:

Reagent	5 mM Tris (uL)
0.05% STEB	9X1000+500
RNase	190
900 mM $MgCl_2$	20
900 mM $CaCl_2$	20
WS-A	2X1000+820
WS-B	2X1000+820
WS-C	2X1000+940
RNA	990+495
DNA	998

30 Add **900 µl** Tris buffer ([M] **5 mM** , pH **8.0**) to each tube in Set 1

31 Add Tris buffer ([M] **5 mM** , pH **8.0**) to the tubes in Set 2. The unit of volume is uL.

Tubes A	650	640	625	600	550	500	640	630	610	580	550
Tubes B	650	640	625	600	550	500	640	630	610	580	550
Tubes C	600	590	575	550	500	450	590	580	560	530	500

32 Add Tris buffer ([M] **5 mM** , pH **8.0**) to tubes in Set 3. The unit of volume is uL.

Tubes A	650	650	650	650	650	650	650	650	650	…	650
Tubes B	650	650	650	650	650	650	650	650	650	…	650
Tubes C	600	600	600	600	600	600	600	600	600	…	600

33 Prepare STEB ([M] **0.05 %**)

33.1 Add **500 µl** STEB ([M] **1 %**) to 0.05% STEB tube, and vortex.

34 Add **250 µl** STEB ([M] **0.05 %**) to Set 2.

35 Place RNase, DNase primary stock, RNA primary standard, DNA primary standard and samples **On ice** .

36 Turn on refrigerated centrifuge and set the temperature to **4 °C** .

37 Turn on shaker/incubator and set temperature to **37 °C** .

38 Prepare **900 mM** $MgCl_2$

38.1 Pour **1 M** $MgCl_2$ solution into 5 mL RNase free Falcon tube

38.2 Transfer **180 µl** **1 M** $MgCl_2$ solution into 900 mM $MgCl_2$ tube

39 Add **60 µl** **900 mM** $MgCl_2$ to WS-A and WS-B

40 Prepare **900 mM** $CaCl_2$

40.1 Pour **1 M** $CaCl_2$ solution into 5 mL RNase free Falcon tube

40.2 Transfer **180 µl** **1 M** $CaCl_2$ solution into 900 mM $CaCl_2$ tube

41 Add **60 µl** **900 mM** $CaCl_2$ to WS-A and WS-B

42 Prepare RNase working solution **0.5 mg/ml**

Add **10 µl** RNase primary stock (**10 mg/ml**) to RNase tube

43 Add **60 µl** **0.5 mg/ml** RNase to WS-B and WS-C, keep WS-B and WS-C **On ice** .

44 Add **60 µl** DNase primary stock (**5 mg/ml**) to WS-A, keep WS-A **On ice** .

45 Centrifuge extracted samples **10000 x g, 4°C 00:04:00**

46 Prepare RNA secondary standard **2 ug/ml**

Add **15 µl** RNA primary standard to RNA standard tube and vortex. Keep **On ice** .

47 Prepare DNA secondary standard **1 ug/ml**

Add **2 µl** DNA primary standard to DNA standard tube and vortex. Keep **On ice** .

48 Load **50 µl** WS-A to Tubes A in Set 2 and Set 3.

49 Load **50 µl** WS-A to Tubes C in Set 2 and Set 3.

50 Load **50 µl** WS-B to Tubes B in Set 2 and Set 3.

51 Load **50 µl** WS-C to Tubes C in Set 2 and Set 3.

52 Add **100 µl** centrifuged samples to its corresponding tubes in Set 1. Vortex each tube.

53 From Set 1, transfer **250 µl** of diluted samples to each corresponding tubes in Set 3.

54 Add RNA and DNA secondary standards to Set 2 tubes. The unit of volume is uL.

Tubes A	0	10	25	50	100	150	10	20	40	70	100
Tubes B	0	10	25	50	100	150	10	20	40	70	100
Tubes C	0	10	25	50	100	150	10	20	40	70	100

55 Vortex each tube for **00:00:02** and place all tubes into the shaker/incubator at **37 °C** , continuously shaking at 200 RPM for **00:20:00** . 20m

Fluorescence reading

56 Prepare SYBR Green II working solution (SG-II WS)

56.1 Each 96-well microplate requires 1 mL of SG-II WS.

56.2 Wrap 5 mL tube with foil, add **7 µl** SYBR Green II 10,000X concentrate to each one mL Tris buffer (**5 mM** , **8.0**) in biosafety cabinet.

56.3 Load **10 µl** SG-II WS to each well in the microplate with 0.5 mL tip of stepper.
Adhere black film on the top of a microplate lid and cover the plate with this lid.

57 After incubation, stop reaction by vortexing each tube for **00:00:02** and then place into the fridge.

58 Allow samples to reach **Room temperature** for **00:02:00** before loading the microplate.

45m

59 Load **190 µl** working sample to the microplate.
Standards must be included in each microplate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	R1A	R2A	R3A	R4A	R5A	R6A	1A	1A	1B	1B	1C	1C
B	R1B	R2B	R3B	R4B	R5B	R6B	2A	2A	2B	2B	2C	2C
C	R1C	R2C	R3C	R4C	R5C	R6C	3A	3A	3B	3B	3C	3C
D	D1A	D2A	D3A	D4A	D5A		4A	4A	4B	4B	4C	4C
E	D1B	D2B	D3B	D4B	D5B		5A	5A	5B	5B	5C	5C
F	D1C	D2C	D3C	D4C	D5C		6A	6A	6B	6B	6C	6C
G	9A	9A	9B	9B	9C	9C	7A	7A	7B	7B	7C	7C
H	Blank A	Blank A	Blank B	Blank B	Blank C	Blank C	8A	8A	8B	8B	8C	8C

10m

60 **Room temperature** Shake black film covered microplate for **00:10:00**

61 Setup microplate reader:

Plate: Greiner F bottom chimney well PP 96 well;

Endpoint reading: Ex 490 nm/Em 520 nm;

Shake: Continuous 5s at 600 rpm

62 Read fluorescence and export data to excel sheet.

Standard curve and calculation

63 RNA standard curve

63.1 Concentrations of RNA standards in the microplate

Standard	2 ug/mL (uL)	Tris+WS (uL)	0.05% STEB (uL)	SG II WS (uL)	Final (ng/mL)
R1	0	700	250	50	0
R2	10	690	250	50	20
R3	25	675	250	50	50
R4	50	650	250	50	100
R5	100	600	250	50	200
R6	150	550	250	50	300

63.2 Slope of fluorescence in Tube A vs concentration of RNA standard gives $m_{RNA+DNase}$
Slope of fluorescence in Tube B vs concentration of RNA standard gives $m_{RNA+RNase}$

63.3 Calculate ρ

$$\rho = \frac{m_{RNA+RNase}}{m_{RNA+DNase}}$$

64 Total RNA of the samples

$$ug\ Total\ RNA\ (ml\ assay)^{-1} = 0.001 \times \frac{(RFU_A - RFU_{A_{Blank}}) - (RFU_C - RFU_{C_{Blank}})}{(1-\rho)} / m_{RNA+DNase}$$

Where,
RFU_A and RFU_C are the fluorescence in Tube A and Tube C of the same sample.
RFU_{ABlank} and RFU_{CBlank} are the fluorescence in Tube A and Tube C of the blank.

65 DNA standard curve

65.1 Concentrations of DNA standards in the microplate

Standard	1 ug/mL (uL)	Tris+WS (uL)	0.05% STEB (uL)	SG II WS (uL)	Final (ng/mL)
D1	10	690	250	50	10
D2	20	680	250	50	20
D3	40	660	250	50	40
D4	70	630	250	50	70
D5	100	580	250	50	100

65.2 Slope of fluorescence in Tube A vs concentration of DNA standard gives $m_{DNA+DNase}$
Slope of fluorescence in Tube B vs concentration of DNA standard gives $m_{DNA+RNase}$

65.3 Calculate δ

$$\delta = \frac{m_{DNA+DNase}}{m_{DNA+RNase}}$$

66 Total DNA of the samples

$$ug\ Total\ DNA\ (ml\ assay)^{-1} = 0.001 \times \frac{(RFU_B - RFU_{B_{Blank}}) - (RFU_C - RFU_{C_{Blank}})}{(1-\delta)} / m_{DNA+RNase}$$

Where,
RFU_B and RFU_C are the fluorescence in Tube B and Tube C of the same sample
RFU_{BBlank} and RFU_{CBlank} are the fluorescence in Tube B and Tube $_C$ of the blank.

67 Dilution factor=40
If,

- Sample is extracted by 1 mL extraction reagent
- In Set 1, sample is diluted to 1/10
- In Set 3, diluted by Tris and all working solutions to 250/950
- In microplate, diluted by SG-II WS to 190/200