RNA extraction from Synechocystis sp. PCC 6803 with Trizol reagent

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Abstract

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Protocol

Step 1.
Label all required tubes and store on ice (or freezer, if not needed immediately)

Step 2.
Pre-cool all required centrifuges

Step 3.
Fill sterile 50 mL tube (Falcon) w/ ice and store in ice bath

Step 4.
Pour 20-25 mL cell culture (OD750 < 1.0) to ice-filled tube (up to 45 mL mark)Note: avoid long transport of cell culture before harvest

Step 5.
spin down at 4000 - 5000 g and 4 ° C for 5 min

Step 6.
discard supernatant (w/ ice) into big beakerNote: depending on strain/mutant some cells will get lost at this step

Step 7.
resuspend cell pellet in residual water (1 mL)

Step 8.
transfer suspension into 2 mL (safe lock!) tubes (work on ice!)

Step 9.
spin down at 13.000 g and 4° C for 15 sec

Step 10.
discard supernatant w/ pipetNote: try to remove supernatant 'as quantitatively as possible'

Step 11.
resuspend pellet in 1 mL Trizol reagent

Step 12.
store at -20 °C or (better) -80 °C

Step 13.
incubate frozen samples at 65° C for 15 min under constant agitationNote: if no shaking thermoblock is available, vortex once per minute

Step 14.
add 200 µL (ice cold) chloroform-isoamylalcohol (24:1) per 1 mL Trizol and vortex for 30 sec

Step 15.
spin down at 11.000 g and 4° C for 10 min

Step 16.
transfer upper, aqueous phase to fresh 1.5 mL tube

Step 17.
add 1 Vol. (ice cold) phenol-chloroform-isoamylalcohol (25:24:1)Note: for RNA preparation phenol solution/mixtures should not be Tris-buffered

Step 18.
transfer upper, aqueous phase to fresh 1.5 mL tube

Step 19.
add 1 Vol. isopropanol (2-propanol), 10 µL 3 M Na-Acetat (pH 5.2) and 1 µL glycogen (RNA grade, Thermo)

Step 20.
incubate o/n at -20 for precipitation

Step 21.
spin down at 13.000 g and 4° C for 30 min

Step 22.
remove supernatant and wash pellet w/ 70% EtOH (ice cold)

Step 23.
spin down at 13.000 g and 4° C for 10 min

Step 24.
repeat steps 24 and 25

Step 25.
discard supernatant and air-dry RNA pellet for 10 min

Step 26.

resuspend RNA Pellet in 30 µL ultra-pure water and store at -20 or (better) -80°C