ITQB-Nova - Universidade Nova de Lisboa	EPP-SOP-ITQB01
	Version 1.0

EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Extraction of total RNA from *P. putida*

Total RNA extraction for downstream applications

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Instruction

Extraction of total RNA from *P. putida*

1 Introduction / Purpose

This protocol describes the method for total RNA extraction from *Pseudomonas putida.* RNA obtained from this method ranges from large transcripts to small RNA molecules. It is not completely DNA free, so Real-Time PCR downstream applications need further RNA sample treatment with a DNase.

2 Equipment and chemicals

2.1 Equipment

- ⇨ Centrifuge
- ⇨ Water bath
- ⇨ Tissue lyser (FastPrep)

2.2 Chemicals

Tris base
Lysozyme
Glass beads
Acid phenol (for RNA)
DNase RNase free (Turbo DNase Ambion)
Trizol
$CHCl_3$
Isoamyl alcohol
isopropanol
Ethanol (EtOH)

2.3 Bacterial strains

This protocol can be applied to any P. putida strain

3 Media and buffers

Lysis solution: Weight 20 mg of lyophilized powder lysozyme and mix in 10 ml of Tris-Cl buffer 10mM pH7.5. Aliquot the solution and store -20 °C.

4 Procedures

4.1 Cell Lysis

Resuspend the collected pellets into 350 µl of lysis solution in 2 ml tube and incubate for 5 min at 37°C.

Change the suspension to a 2ml eppendorf with screw cap containing 0.5ml glass beads (smaller ones) 0.5ml acid phenol (RNA).

Lyse the cells in the Tissue lyser (FastPrep) – speed 6, 45s (2x).

4.2 DNase Treatment

Centrifuge max speed, 10min, 4ºC.

Take the aqueous phase into 2ml eppendorfs

Add 50µl DNase Buffer 10x

Add 10U DNase RNase free (Turbo DNase Ambion). Incubate at 37ºC, 1H.

4.3 RNA extraction

Add 1ml Trizol + 200µl $CHCl_3$

Vigorous agitation 2 min room T

Centrifuge 14000 rpm, 10min, 4ºC

Take the aqueous phase

Add 200µl $CHCl_3$:Isoamyl alcohol (24:1)

Vigorous agitation 2 min room T

Centrifuge 14000 rpm, 15min, 4ºC

Take the aqueous phase

Add 500µl isopropanol

Soft vortex 5 to 10sec

10min at room T

Centrifuge 14000 rpm, 8min, 4ºC

Wash with EtOH 70%

Centrifuge 14000 rpm, 5min, 4ºC

Dry at 37ºC ~15min

Resuspend in 100µl H_2O (let hydrate before resuspending)

5 *remarks/troubleshooting:*

6 *Biosafety*

Work in a fume hood

Wear protective gloves and lab coat.

7 *Acknowledgements*

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