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Preferential Lysis of S. rosetta for Total RNA (VERSION 2)

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Protocol status: Working
We use this protocol and it's working

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Abstract

Sterol-based detergents, like digitonin more effectively disrupt membranes with sterols, like those of eukaryotes. This protocol leverages the different membrane compositions of eukaryotes and bacteria to preferentially lyses the eukaryotic membranes of the choanoflagellate species, *S. rosetta*, from a co-culture with bacteria. The lysis buffer includes RNase inhibitors to preserve RNAs for RNA purification or cDNA synthesis, and the included protease inhibitors make this protocol suitable for protein extractions for western blots.

Prepare Lysis Buffer

1 Combine the following components for the lysis buffer:

	A	B	C	D	E
	Chemical	**[Final]**	**[Stock]**	**Final Vol**	**Chemical Vol**
	Water			10 ml	0.41 ml
	Tris-HCl, pH 8.0	20 mM	1 M		200 µl
	KCl	150 mM	2 M		750 µl
	MgCl2	5 mM	1 M		50 µl
	Sucrose	250 mM	1.75 M (60% w/v)		1ml 420 uL
	Cycloheximide	100 ug/ml	100 mg/ml		10 ul
	Protease inhibitor tablet	2 mini tablet / 10 ml			1 ml
	Digitonin	10 mM	20 mM		5 ml
	Sodium Heparin	1 mg/ml	100 mg/ml		100 µl
	Pefabloc SC	1 mM	200 mM		50 µl
	DTT	*1 mM*	*1 M*		*10 µl*
	Turbo DNase	*0.1 U/ml*	*2 U/µl*		*500 µl*
	SUPERaseIN	*1 U/ml*	*20 U/µl*		*500 µl*

Notes:
1. Prepare the buffer ahead of time by combining all but the italicized reagents (DTT, Turbo DNase, and SUPERaseIn), splitting into 449.5 µl aliquots, and storing at -20°C.

2. Just before use, thaw the prepared lysis buffer on ice and then add 0.5 µl of 1 M DTT, 25 µl of Turbo DNase, and 25 µl SUPERaseIn to the 449.5 µl aliquot for a total volume of 500 µl.

Count Cells

2 Determine the total number of cells in the culture that will be harvested using a hemocytometer.

2.1 Fix 200 µl of cells with 2 µl of 37% formaldeyde and vortex well.

2.2 Pipet up and down to homogenize cells, and pipet 12 µl of cells into the chamber of a hemocytometer.

2.3 Count the number of cells (N) in the four corner quandrants of a Neubauer, bright-line hemocytometer.

2.4 Calculate the cell concentration ($[Cells]$cells/ml) according to this equation:
$[Cells] = N/4$•10000cells/ml

2.5 Determine the volume of the culture (Vol) and then calculate the total number of cells in the culture ($Total$) according to this equation:
$Total = [cells]$•Vol cells

2.6 Calculate the volume of lysis buffer ($LysisBuffer$ µl) to add to the cells for lysis:
$LysisBuffer = Total/100000$ µl

Harvest Cells

3 Harvest cells of S. rosetta.

3.1 Centrifuge the cells in 50 ml conical tubes at 2400 x g, 4°C, 00:05:00

3.2 Gently remove supernatant with a serological pipette, leaving a small amount of liquid of the pellet.

3.3 Gently remove the remaining supernatant with a fine-tip transfer pipet.

Lyse Cells

4 Lyse cells in preferential lysis buffer.

4.1 Resuspend the cell pellet (go to step #3.3) in the calculated volume of $LysisBuffer$ (go to step #2.6).

4.2 Pipet the cells gently up and down and then incubate On ice for 00:10:00 .

4.3 Homogenize the lysate by passing 5 times through a 30G needle attached to a luer lock syringe.

4.4 Clarify the lysate by centrifugation 6000 x g, 4°C, 00:10:00 10m

4.5 Separate the S. rosetta lysate from the bacterial pellet by using a gel-loading tip to gently transfer the supernatant into a new tube. Pay attention not to disturb the pellet.

Storage

5 Flash freeze lysate in liquid nitrogen and store at -80°C for long term storage.