

## RNA ISOLATION

Lyse  $1 \times 10^8$  cells in 1 mL Trizol.

Lysate can be stored at  $-20^{\circ}\text{C}$ .

Spin lysate at  $12000 \times g$ , 10 min,  $4^{\circ}\text{C}$ .

Keep supernatant and incubate 5 minutes at RT.

Add 200  $\mu\text{L}$  chloroform.

Vortex.

Incubate 3 min at RT.

Spin at  $12000 \times g$ , 15 min,  $4^{\circ}\text{C}$ .

Keep aqueous layer (top).

Add 0.5 mL isopropanol and incubate 10 minutes at RT.

Spin  $12000 \times g$ , 10 minutes,  $4^{\circ}\text{C}$ .

Wash pellet with 1 mL 75% EtOH (in DEPC-water). Vortex.

Spin  $7500 \times g$ , 5 minutes,  $4^{\circ}\text{C}$ .

Air dry 5 minutes.

Dissolve RNA in 500  $\mu\text{L}$  DEPC-water.

Incubate at  $55^{\circ}\text{C}$  for 10 minutes.

Store at  $-80^{\circ}\text{C}$ .