

Isolation of total RNA from 96 well plate

(Simplified protocol from Qiagen RNeasy 96 Hand book)

- 1. Lysis cells directly in 96 well plate by following steps:**
 - a) Aspirate the media from confluent plate of ES clones.
 - b) Wash the wells with 200 μ l of PBS. Aspirate off the PBS completely (incomplete removal of the supernatant will lead to reduced yield).
 - c) Add 150 μ l/well of RNA lysis buffer (RLT) (10 μ l of β -mercaptoethanol per 1ml of RLT must be added before use).
 - d) Keep the plate flat on the bench, shake it vigorously back and forth for 10 sec. While continuing to keep the plate flat on the bench, rotate the plate by 90° and shake it for an additional 10 sec.

Now you can cover the plate with a foil Costar cover, label it and freeze at -86° for late isolation, or go to step 2 to do it right away.
- 2. Add 1 volume (150 μ l) of 70% ethanol to each well. Mix by pipetting up and down 3 times.**
- 3. Place an RNeasy 96 plate on top of a Square-well Block. Mark the plate for late identification.**
- 4. Apply the samples from step2 into the wells of the RNeasy plate. Seal the plate with tape.**
- 5. Load the Square-well Block and RNeasy 96 plate into holder. Centrifuge at 6000 rpm for 4 min at room temperature (RT).**
- 6. Add 0.8 ml of buffer RW1 to each well of the RNeasy 96 plate. Seal the plate with a new piece of tape.**
- 7. Centrifuge at 6000 rpm for 4 min at RT.**
- 8. Place the RNeasy 96 plate on top of another clean Square-Well Block. Add 0.8ml of buffer RPE to each well. Seal the plate. Spin at 6000 rpm for 4 min at RT.**
- 9. Add another 0.8 ml of Buffer RPE to each well. Seal it. Spin at 6000 rpm for 10 min at RT.**
- 10. Place the RNeasy 96 plate on top of a collection microtube rack containing 1.2 ml collection microtubes.**
- 11. To elute the RNA, add 45-70 μ l (we use 60 μ l) of RNase-free water to each well (make sure to pipet the water directly onto the membrane). Seal it and incubate for 1 min at RT. Then centrifuge at 6000 rpm for 4 min at RT. Store the total RNA at -86C.**