NucleoZOL Total RNA Isolation Protocol

(Xinyi Yu, commented by TCH @ 08/21/2017)

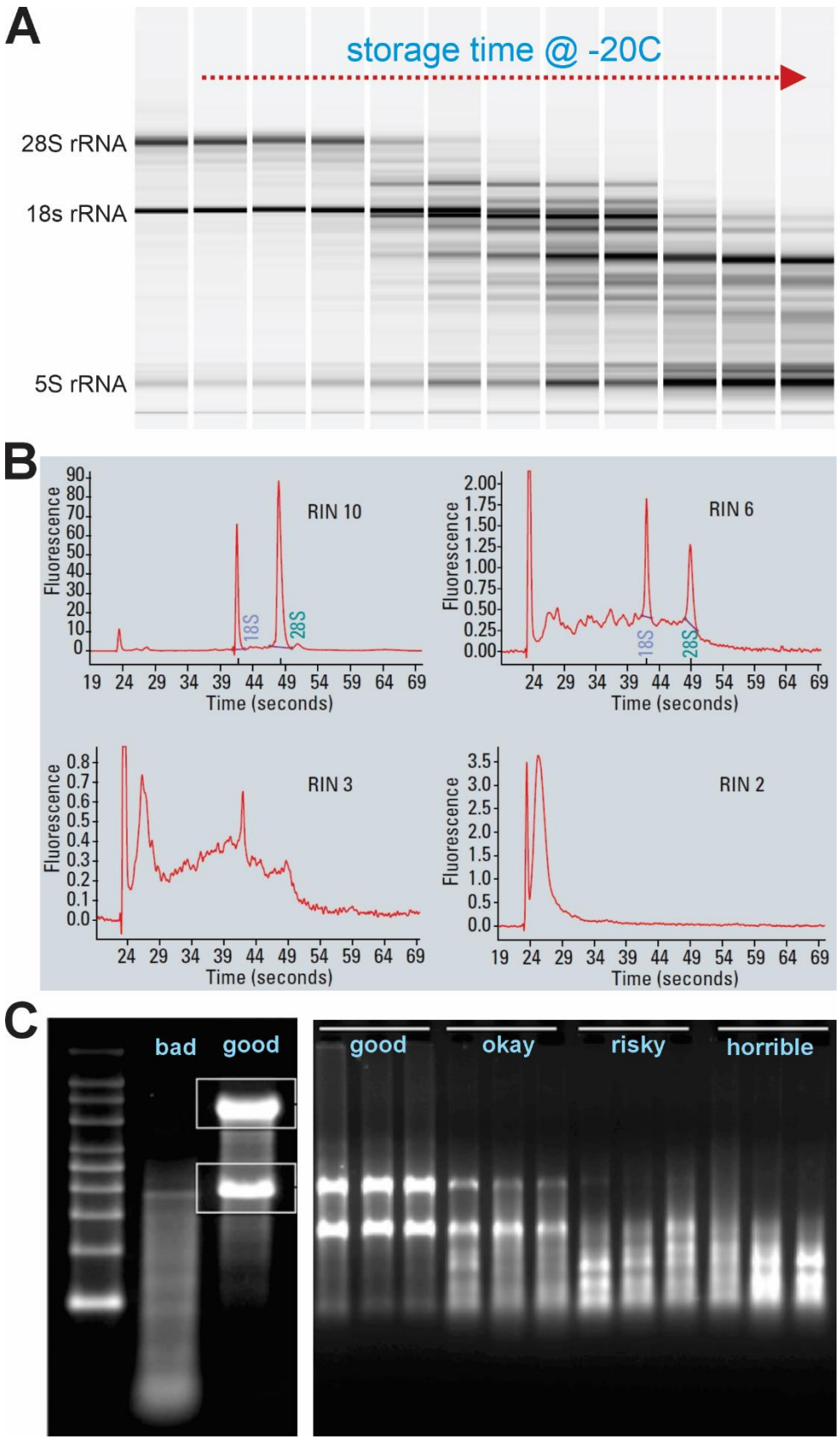
NucleoZOL Reagent, Cat# 740404.200 (Takara BIO, USA)

1) **LYSE CELLS:** For cells grown in monolayer, remove cell culture medium and lyse cells by addition of **1mL of NucleoZOL** to the culture disk (e.g., **1ml per one confluent 100mm dish; or 500µl per 60mm dish**). For cells grown in suspension, spin down cells and lyse directly by the addition of NuceoZOL at **1mL NucleoZOL/10^7 cells**. Lyse cells by pipetting up and down several times. Ensure complete lysis by repeated pipetting.
2) **PRECIPITATE PROTEINS/CELL DEBRIS:** Add **200µL** RNase-free water per 500uL NucleoZOL to the lysate. Shake the sample vigorously for 15 seconds. Centrifuge samples for 5~8min at 12,000 x g at 4°C or room temperature.
3) **PC-8 EXTRACTION:** Transfer the RNA-containing supernatant to 2.0ml-Eppendorf tubes, add equal volume of PC-8, vortex vigorously, and centrifuge the mix at top speed for 3-5min. If visible precipitates appear at the interphase, repeat the PC-8 extraction till the interphase is clear. [**NOTE**: while this step is optional, it is strongly recommended to avoid any significant RNA degradation, especially when RNA is isolated from tissue samples. PC-8 extraction will remove RNase completely].
4) **PRECIPITATE TOTAL RNA:** Carefully pipette RNA containing top phase from **Step 3** into a 1.7ml or 2.0ml RNase-free Eppendorf tube(s). Add **1mL isopropanol** per **1mL supernatant** (**i.e., equal volume**) in order to precipitate RNA. Make sure tubes are kept on ice. Centrifuge samples for **5~8min** at **12,000 x *g*** at 4°C or room temperature. (**NOTE**: You can stop here by storing the isopropanol-RNA mix at -80°C for days to years).
5) **WASH RNA:** Use **400–600µL 70 % ethanol** when precipitating in 1.5mL or 2mL tubes. For larger tubes, add 500-700uL 70 % ethanol per 1mL supernatant. Centrifuge the pellets for 1~3 min at 12,000 x *g*.
6) Remove ethanol from the pellet by carefully pipetting. Repeat the ethanol washing step. Do not dry the RNA pellet between wash steps.
7) **RECONSTITUTE RNA:** Dissolve the RNA pellet in RNase-free water to obtain an RNA concentration of 1~2µg/µL (i.e., usually dissolve in 20~30µl for one 100mm dish, or 10~15ul for one 60mm dish). Vortex the sample for 2~5min at room temperature to dissolve total RNA. Use **1~2µl RNA** sample to run RNA "bleach gels" to check the quality/integrity of the sample (see **Protocol C12 and see the images below**). Typically, use **5~10µg RNA** per RT-PCR reaction.
8) Your RNA samples should be aliquoted and kept at -80°C (not your own -20°C freezer space).

NOTE: Similar to TRIzol reagent, NucleoZOL reagent contains a very low level of quinidine to inhibit RNase activity. Thus, you should take the following considerations in mind:

1). You should try to collect one sample at a time (keep the rest in the incubator). Once you lyse the cells, please collect the lysate and transfer to 2ml-Eppendorf tubes. Please keep the tubes on ice.

2). The RNA will severely degrade if the cell lysate is kept in -20°C or -80°C. You should process the samples all the way to isopropanol precipitation/ethanol washes (even though you may leave the isopropanol-filled tubes at -80°C for weeks/months).



Assessment of RNA Quality and Integrity Is Essential for Downstream Analyses (esp. RT-PCR and qPCR). **(A)** A total RNA sample was degraded for varying times at -20C and the resulting samples were analyzed on the Agilent 2100 Bioanalyzer System using the Eukaryote Total RNA Nano assay. A shift towards shorter fragment sizes can be observed with progressing degradation. **(B)** Sample electropherograms used to train the RNA Integrity Number (RIN) software. Samples range from intact (RIN 10), to degraded (RIN 2). **(C)** Representative "good" and "bad" RNA samples.