Metabolic Labeling of rRNA with $^{32}P_i$

Modified from Pestov et al 2008, Current Protocols in cell biology

1. Plate cells in 6-well dish 1 day before experiment. For U2OS cells, plate 3 x 10^5 cells.
2. One hour before starting labeling reaction, replace cell growth media with 1.0 ml of prewarmed phosphate-free media (ThermoFisher Gibco 11971025) with dialyzed FBS (ThermoFisher Gibco 26400044).
3. Typically, if drug treating, add drugs to phosphate free media after 50 minutes of incubation.
4. Add 10 μCi/ml of $^{32}P_i$ to media and incubate for 1 hour. Source radiation from Perkin-Elmer (NEX053H002MC) is 10 μCi/μl, thus, to the 1 mL of media, add 1 μl of $^{32}P_i$.
5. After 1 hour, remove $^{32}P_i$ containing media.
6. Wash 1x with prewarmed DMEM.
7. Add prewarmed DMEM. Make sure to add drugs if you intend on monitoring drug treatment.
8. At desired time-points, remove media, wash 1x with PBS
9. Add 0.5 mL of Trizol (Invitrogen) and extract with 100 μl of chloroform
10. Ethanol precipitate
11. Resuspend in 33 μl of RNA loading dye.
12. Run 10 μl on 1.2 % Hepes-EDTA-Formaldehyde gel overnight at 65V.
13. Dry gel and expose to film.

10X HEPES-EDTA Buffer

200 mM HEPES

10 mM EDTA

Adjust pH to 7.8

RNA loading buffer (make fresh)

Deionized formamide (95%)	7 μl
Formaldehyde (40%)	2 μl
10X H-E Buffer	1 μl
EtBr (400 μg/ml)	1 μl
0.5% Bromophenol Blue	1 μl