RNA precipitation

Materials:

1. RNase-free tubes and filter tips
2. The product from the in vitro transcription
3. Ice cold absolute EtOH
4. Ice-cold 70% EtOH
5. Ice-cold 3M ammonium acetate

Method:

1. Prepare the absolute, the 70% EtOH and the 3 M ammonium acetate the day before the precipitation and leave them -20^{0}C.
2. For the precipitation of the RNA from the in vitro transcription add the pre-chilled 108 µL ice-cold 3 M ammonium acetate[1] and the pre-chilled 750 µL absolute ice-cold EtOH. Leave for 2 to 24h at -80^{0}C[2].
3. After the incubation centrifuge for 1h at 4^{0}C[3]at 14 200 rcf (Relative centrifugal force)
4. After the centrifugation is over, removing the supernatant by pipetting[4]
5. Then wash with 500 µL 70% of ice-cold EtOH to remove residual salt.
6. Again, remove the supernatant
7. Leave the Eppendorf tubes to air-dry for 3-5 min [5] so that the remaining alcohol can evaporate.
8. Add 50 µL of nuclease free water to resuspend the RNA pallet.
9. Use the Nanodrop to quantify the amount of DNA
10. After you have quantified of your RNA with Nanodrop you should add appropriate amount of Hub-mix to the RNA by the formula:

$$\frac{ng/\mu\text{L of the RNA x 50 }\mu\text{L}}{100ng} - 50 = \text{Volume of Hub} - \text{mix to add}$$

The result after the formula should be 100ng/ µL of RNA.

Notes:

1. Use the 5 M Ammonium Acetate stock solution and bring the final concentration of ammonium acetate to 3 M.
2. Longer incubation times at lower temperatures may result in better recovery of nucleic acids.
3. If you do not have a centrifuge that can reach 4^0C you can put your centrifuge in the refrigerator and do the procedure, there.
4. At this step at the bottom of the Eppendorf tube will be visible a small white pallet which represents the precipitated RNA. Be careful not the pipette it during the removal of the supernatant.
5. Do not over-dry because it will be difficult afterwards to dissolve the RNA pallet with nuclease free water.