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mRNA Synthesis Protocol using the HiScribe T7 mRNA Kit with CleanCap Reagent AG V.1

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for mRNA preparation

Standard mRNA Synthesis Protocol using the HiScribe T7 mRNA Kit with CleanCap Reagent AG (NEB #E2080)

2h 15m

1 BEFORE STARTING

- Recommend wearing gloves and using nuclease-free tubes and reagents to avoid RNase contamination. Reactions are typically 20 µl but can be scaled as needed.
- Thaw the necessary kit components to room temperature (Reaction Buffer, NTPs, CleanCap Reagent AG and Template DNA), mix and microfuge briefly to collect solutions to the bottom of the tubes. Keep the Enzyme Mix on ice.
- If several reactions will be performed a master mix of Reaction Buffer, NTPs and CleanCap can be prepared according to the volumes per reaction below. Use 12 µl of the Master Mix per reaction.

2 1 - Set up the following reaction at room temperature in the following order: 2h 15m

2 - Gently mix the reaction by pipetting up and down and microfuge briefly. Incubate at 37 °C for 02:00:00 .

For reaction times of 60 minutes or less, a water bath or heat block may be used. For reactions longer than 60 minutes, we recommend using a dry air incubator or PCR instrument set to 37 °C *(with a heated lid) to prevent evaporation. For reactions with transcripts <0.3 kb incubations up to 16 hours or overnight can be performed.*

3 - Bring the reaction volume up to 50 µL with nuclease-free water. Add 2 µL of DNase I, mix well and incubate at 37 °C for 00:15:00 .

4 - Proceed with mRNA purification.

Monarch RNA Cleanup Kit Protocol (after IVT) NEB #T2030

3m

3 BEFORE STARTING 3m

- The standard protocol outlined below will purify RNA ≥ 25 nt. A simple modification in Step 2 can allow for the purification of RNA as small as 15 nt.
- Centrifugation should be carried out at 16,000 x g in a standard laboratory microcentrifuge at room temperature.
- Add 4 volumes of ethanol (>95%) to one volume of RNA Cleanup Wash Buffer

1. Add 100 µL Cleanup Binding Buffer to the 50 µL sample. A starting sample volume of 50 µL is recommended. For smaller samples, nuclease-free water can be used to adjust the volume. For samples larger than 50 µL, scale buffer volumes accordingly. Samples with a starting volume > 150 µL will require reloading of the column during Step 3.
2. Add 150 µL (1 volume) of ethanol (≥ 95%) to your sample and mix by pipetting or flicking the tube. Do not vortex. This will enable the binding of RNA ≥ 25 nt. If you wish to bind RNA as small as 15 nt, add 2 volumes (300 µL) of ethanol to your sample instead of 1 volume (150 µL). The addition of 2 volumes of ethanol shifts the cutoff size of RNA binding from 25 nt down to 15 nt.
3. Insert column into collection tube, load sample onto column and close the cap. Spin for 00:01:00, then discard flow-through. For diluted samples > 900 µL, load a portion of the sample, spin, and then repeat as necessary.
4. Re-insert column into collection tube. Add 500 µL Cleanup Wash Buffer and spin for 00:01:00. Discard the flow-through.
5. Repeat wash (Step 4).
6. Transfer column to an RNase-free 1.5 mL microfuge tube (not provided). Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 00:01:00 to ensure traces of salt and ethanol are not carried over to the next step.
7. Elute in 20 µL nuclease-free water. The eluted RNA can be used immediately or stored at -80 °C. Care should be used to ensure the elution buffer is delivered onto the matrix and not the wall of the column to maximize elution efficiency.

Lopez Lab Specifics: Characterization: To check QC (size and degradation) and

endotoxins.

4 For QC
https://research.wustl.edu/core-facilities/genome-technology-access-center/
Person in charge: Michael Heinz mheinz@wustl.edu
4444 Forest Park Ave. 04140
Online submission form: https://gtac-old.wustl.edu/request/service/index.php
Quantity: 30 ng in 10 µL nuclease free H2O.
Shipment: 1.7-2.0 mL microcentrifuge tubes / dry ice

For Endotoxins
https://research.wustl.edu/core-facilities/biologic-therapy-core-facility-siteman-cancer-center/
Person in charge: Kelsi Rotter krotter@wustl.edu
Southwest Tower 00719
Quantity: 500 ng in 150 µL nuclease free H2O.
Shipment: 1.5 mL microcentrifuge (new plasticware certified to be pyrogen free) / dry ice