SIGNALCHEM DIAGNOSTICS

190-13160 Vanier Pl, Richmond,
B. C., Canada, V6V 2J2
+ 1 778 326 0223
info@signalchemdx.com
www.signalchemdx.com

INSTRUCTIONS FOR USE

Product Name: T7 High-Yield RNA Synthesis Kit **Catalog # T702-K598**

DNA Template preparation: Linearized plasmids with T7 promoters or PCR products can serve as templates. Resuspend in TE buffer or RNase-free H_2O. T7 Promoter Sequence: TAATACGACTCACTATAG*GG (*G = transcription start site).

A) Plasmid Template:

Step 1. Insert the target DNA into a plasmid vector containing the T7 promoter.

Step 2. Linearize completely using restriction enzymes and purify.

- Circular plasmids yield heterogeneous RNA; linearization ensures specific-length transcripts.
- Use restriction enzymes that cut downstream of the insert (5′ overhangs or blunt ends preferred).
- Purify linearized plasmids to remove enzymes/salts that may inhibit transcription.

B) PCR Product Template:

Step 1. Add the T7 promoter sequence to the 5′ end of the upstream primer.

Step 2. Amplify using high-fidelity polymerase.

- Confirm PCR product specificity/concentration via electrophoresis. Use 2–5 µL per 20 µL reaction.
- Gel purification improves RNA yield and quality.

In Vitro Transcription:

Step 1. Centrifuge T7 RNA Polymerase Mix briefly; keep on ice.

Step 2. Thaw 10× Transcription Buffer and NTPs; vortex and spin down.

Step 3. Setup reaction (20 µL System):

2 µL	10× Transcription Buffer
2 µL each (10 mM each)	ATP/CTP/GTP/UTP (100 mM each)
1 µg	DNA Template
2 µL	T7 RNA Polymerase Mix
Up to 20 µL	RNase-free H_2O

- Assemble at room temperature (spermidine in buffer may precipitate DNA at low temps).
- For >1000nt transcripts: Prefer linearized plasmids.
- Use a PCR machine with an open lid to prevent evaporation.
- White precipitate (magnesium pyrophosphate) is harmless; add EDTA if needed.

Step 4. Mix, spin down, and incubate at 37°C for 2 hours (extend to 4–8 hours for short <100nt transcripts).

Step 5. (Optional) Add 2 µL RNase-free DNase I and incubate at 37°C for 15 min to remove DNA template.

RNA purification:

A) RNA Cleaner Magnetic Beads:

Step 1. Equilibrate RNA Clean Beads to room temperature (~30 min).

Step 2. Dilute transcription product to 50 µL with RNase-free H_2O.

Step 3. Add 100 µL beads (2× volume), pipette mix 6×.

Step 4. Incubate 5 min at RT for RNA binding.

Step 5. Place on magnetic stand for 5 min; discard supernatant.

Step 6. Wash twice with 200 µL freshly prepared 80% ethanol (30 sec per wash).

Step 7. Air-dry beads 5 min (avoid cracking).

Step 8. Resuspend in 22 µL RNase-free H_2O; incubate 5 min.

Step 9. Transfer 20 µL supernatant to a new tube (avoid beads).

Step 10. Store at –80°C if not used immediately.

B) Phenol/Chloroform Extraction:

Step 1. Dilute 20 µL reaction with 115 µL H_2O + 15 µL 3M NaOAc (pH 5.2).

Step 2. Extract with 1× phenol/chloroform (1:1) ⟶ 2× chloroform.

Step 3. Add 2× volume ethanol; incubate ≥30 min at –20°C.

Step 4. Centrifuge 15 min (max speed, 4°C); wash pellet with 500 µL 70% ethanol.

Step 5. Resuspend in 20 µL RNase-free H_2O; store at –80°C.

C) Lithium Chloride Precipitation (for RNA length >300 nt and ≥100 ng/µL):

Step 1. Add 30 µL H_2O + 30 µL 7.5M LiCl to 20 µL reaction.

Step 2. Incubate ≥30 min at –20°C; centrifuge as above.

Step 3. Wash pellet with 70% ethanol; resuspend in 20 µL H_2O.

D) Column Purification:

Dilute product to 100 µL with RNase-free H_2O; follow column protocol.

RNA Quantification:

UV Absorption: Measure A260/A280.

Dye-Based (RiboGreen): Accurate for purified/unpurified RNA (unaffected by free nucleotides).

RNA Quality Assessment:

Gel Electrophoresis: Use agarose or polyacrylamide gels to check size/integrity.

Agilent 2100 Bioanalyzer: Requires minimal RNA; high-quality samples show sharp peaks.

Notes:

- Use RNase-free tips/tubes and avoid RNase introduction during handling.