–80°C. Usually, 3–5 mL of SYNV polymerase extract is obtained from 100 g of tissue using this protocol.

4.3.2. Polymerase Reactions

1. For each reaction mixture, prepare 20 µL of the polymerase extract in a 200-µL reaction. The final concentration of the reactants is 6 m*M* $MgCl_2$, 50 m*M* $(NH_4)_2SO_2$, 12.5 m*M* HEPES, pH 8.0, 2 m*M* DTT, 1 m*M* ATP, 0.5 m*M* CTP, 0.5 m*M* GTP, and 20 µ*M* UTP, plus 50 µCi of [α^{32}P] UTP, 5 U of DNase I, 50 U of RNasin (Promega), and 2% v/v glycerol.
2. Incubate the reactions at 28°C for various periods (*see* **Subheading 4.4., Note 3**).
3. Stop the reactions by adding SDS to 0.5% and EDTA to 5 m*M*. React for 30 min at 42°C with proteinase K (500 µg/mL) to digest the proteins.
4. Extract the RNAs with phenol-chloroform and precipitate by adding 200 µL of 5*M* NH_4Acetate and 1 mL of ethanol to 200 µL of extracted RNA.
5. **Figure 4** shows the results of a slot blot hybridization obtained from transcriptions with purified nuclei and extracted polymerase from uninfected and SYNV-infected plants (*see* **Subheading 4.4., Note 4**).

4.4. Notes

1. **Caution:** DEPC is volatile, so a hood should be used at all times. DEPC can be extremely irritating to the eyes, mucous membranes, and skin when used without ventilation. DEPC can also cause loss of sensation in the fingers and outer extremities when working in areas where high concentrations are allowed to accumulate. The compound is also suspected of being a carcinogen. Therefore, it should always be used in a hood. DEPC reacts with primary amines and also inactivates both proteins and nucleic acids, so direct contact with the agent can destroy the biological or biochemical activity of enzymes and single-stranded nucleic acids. Upon autoclaving, DEPC decomposes to yield ethanol and CO_2. Mixture with ethanol increases the solubility in H_2O, and a 50% solution disperses much more readily in solutions than concentrated DEPC.
2. Most of the nuclei pellet through the 75% Percoll, but the SYNV transcriptase activity in the pelleted nuclei is far lower than that of the nuclei from the 75% interface. From this result, we believe that the nuclei containing the highest amounts of polymerase activity have lower densities than nuclei derived from cells that do not contain actively replicating virus.
3. The polyadenylated leader RNA, and full-length polyadenylated N, M2, sc4, M, and G mRNAs appear in the order of their location on the SYNV genome. By 6 h postinoculation, full-length polyadenylated mRNA products can be detected by selection with oligo(dT) cellulose chromatography.
4. We have also analyzed the polymerase RNA products by a variety of other procedures that are described in Wagner et al. ***(10)*** and Wagner and Jackson ***(20)***.