Additional tRNAs are also supplemented to expand the range of RNAs that are translated. Hemin may also be added to prevent inhibition of translation initiation, because it suppresses an inhibitor of the elongation initiation factor eIF-2a. Potassium and magnesium are also added to a level recommended for translation of most mRNA species. Lysates are treated with micrococcal nuclease to remove any traces of endogenous mRNA that could interfere with subsequent reactions.

Once the translation system has been programmed with the transcript of interest, proteins produced can be detected by incorporation of a radiolabeled ribonucleotide, generally [^{35}S] methionine or cystine, or [^{3}H]leucine. Products may be visualized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by autoradiography. This allows proteins to be sized by comparison with predetermined markers. Being an in vitro system, smaller protein bands are usually present on autoradiographs, caused by premature termination of translation by lysate ribosomes. If specific antibodies are available against the product of an in vitro translation reaction, a further means of identifying and quantifying may be provided; e.g., immunoprecipitation protocols may be employed.

Recently, coupled transcription–translation systems have become available in which circular DNA can be used as template, thereby greatly reducing the workload. Combination systems that consist of a mixture of RRL and WGL may also be used.

2. Materials

2.1. In Vitro Transcription

1. Transcription buffer, usually supplied with the polymerase enzyme being used at either a 10X or 5X concentration (e.g., Promega [Southampton, UK] 5X buffer supplied with the T3 RNA polymerase; *see* **Note 1**).
2. Ribonucleotides diluted to a 10-m*M* concentration (e.g., Pharmacia [Herts, UK] ribonucleotides supplied at 100 m*M*).
3. Dithiothreitol (DTT) at 100 m*M*.
4. RNase inhibitor (e.g., Promega RNasin®, supplied at 33 U/μL).
5. DEPC-treated water (*see* **Note 2**).
6. RNA polymerase enzyme (e.g., Promega T3 RNA polymerase supplied at 5 U/μL).

2.2. In Vitro Translation

1. Commercial in vitro translation system (e.g., Promega RRL or WGL translation systems; *see* **Note 3**).
2. Ribonuclease inhibitor (e.g., Promega RNasin®, supplied at 33 U/μL).
3. [^{35}S] Methionine (1200 Ci/mmol) at 10 mCi/mL (e.g., Amersham, Arlington Heights, IL).
4. DEPC-treated water.