gel, and the mixture is immediately poured between the two glass plates on top of the resolving gel, with the well-forming comb in place. The gel is allowed to polymerize for 30 min.
7. When the gel is ready, remove the well comb and place in the electrophoresis apparatus. Fill with 1X glycine running buffer.
8. To 10 μL of translation reaction, add 10 μL of SDS loading buffer and boil for 2 min to denature proteins.
9. Load sample onto the gel and electrophorese at voltages between 100 and 200 V, until the bromophenol blue dye in the sample buffer has disappeared into the running buffer.
10. The gel is then removed from the apparatus and proteins are stained in Coomassie blue stain for 30 min, and destained for 30 min. This fixes the proteins into the gel.
11. The gel is then dried and exposed to X-ray film (*see* **Note 8**).
12. The size of the in vitro translation products may then be compared with mol-wt markers (radioactive or nonradiocative) run on the same gel (*see* **Note 9**).

3.5. Immunoprecipitation

1. From a typical reticulocyte translation of 50 μL vol, remove 40 μL of the translation reaction to a fresh microcentrifuge tube (save remaining 10 μL as a unprecipitated control), and add 14 μL of an 8% SDS solution, and mix by inverting.
2. Heat the tube at 90°C for 2 min.
3. Add 540 μL of the TNTE buffer and mix gently by inverting the tube several times; allow to cool to room temperature.
4. Add 2 μL of the required antibody and mix the tube by inverting gently.
5. Incubate the tube at 20°C for 2 h, or 4°C overnight, on a rotation platform.
6. Add 2.5 mg of protein A Sepharose.
7. Attach to a rotating platform for 1 h at 20°C.
8. Centrifuge the tube at 1600*g* in a microcentrifuge for 5 min to collect the pellet.
9. Remove all supernatant and wash the pellet once in TNTE and collect the pellet by microcentrifugation.
10. Wash the pellet twice in High-salt TNTE, as in **step 9**.
11. Wash the pellet four times in TNTE, as in **step 9**.
12. Resuspend the final pellet in 20 μL of sample loading buffer, heat to 90°C for 2 min before loading onto SDS-polyacrylamide gels alongside the unprecipitated control from **step 1**.

4. Notes

1. Transcription buffer, either at a 5X or 10X concentration, is supplied with the polymerase enzyme used. This buffer provides the optimal conditions for enzyme activity. 10X buffer for T3 RNA polymerase consists of the following components: 200 m*M* Tris-HCl, pH 8.0, 40 m*M* $MgCl_2$, 10 m*M* spermidine, 250 m*M* NaCl.