3.3. Quantification of Radiolabeled Amino Acid Incorporation

1. 2 μL of the completed translation reaction is removed and is added to 98 μL of 1*M* NaOH/2% H_2O_2 in a 1.5-mL microcentrifuge tube.
2. After vortexing, the tube is incubated at 37°C for 10 min.
3. Translation products are precipitated by adding 900 μL of ice-cold 25% TCA/2% casamino acids and incubating on ice for 30 min.
4. 250 μL of this TCA–reaction mix is precipitated onto a Whatman GF/A glass fiber filter by vacuum filtration.
5. The filter is washed three times with 2 mL of ice-cold 5% TCA and once with 2 mL of acetone. The filter is then allowed to dry completely at room temperature or under a heat lamp.
6. The filter is placed in 2 mL of scintillation fluid, and a value for incorporated counts, given as counts per million (cpm), is obtained in a liquid scintillation counter, on the appropriate setting for the chosen isotope.
7. To determine the total counts present in the reaction, a 5-μL aliquot of the remaining TCA–reaction mix is spotted directly onto a separate glass filter. The filter is allowed to dry and a value for total counts is determined, as for incorporated counts in **step 6** (*see* **Note 6**).
8. Percentage radiolabeled amino acid incorporation is determined as: cpm of washed filter/cpm of unwashed filter × 50.

3.4. SDS-PAGE

1. Glass plates are cleaned, first with detergent and then with ethanol. The gel apparatus is then assembled.
2. Constituents of the resolving gel are mixed in a beaker. These will vary according to the concentration and volume of the gel, but constituent amounts for a 12.5% SDS-PAGE gel in a Bio-Rad vertical minigel apparatus will be provided (20 mL total volume) (*see* **Note 7**).
   a. 6.5 mL Sterile distilled water.
   b. 5.0 mL 1.5*M* Tris-HCl, pH 8.8.
   c. 8.0 mL Acrylamide:*bis*-acrylamide (30:0.8%).
   d. 0.2 mL 10% SDS.
3. Immediately prior to pouring, 50 μL of 10% ammonium persulfate and 5 μL of TEMED are added.
4. The solution is poured between the two glass plates, leaving enough space for the well former and stacking gel. A fine layer of either water or propanol/water is overlayed, and the gel is allowed to polymerize for 60 min.
5. Components for the stacking gel are assembled in a beaker.
   a. 3.05 mL Sterile distilled water.
   b. 1.25 mL 1.5*M* Tris-HCl, pH 8.8.
   c. 0.68 mL Acrylamide:*bis*-acrylamide (30:0.8%).
   d. 0.05 mL 10% SDS.
6. The propanol–water or water layer is poured from the polymerized resolving gel. 25 μL of ammonium persulfate and 2.5 μL of TEMED are added to the stacking