SDS-PAGE protein gel

Materials:

- 20x NuPAGE MOPS SDS running buffer (50 ml)
- Deionised water (1150 ml)
- NuPAGE Bis-Tris pre-cast gel
- NuPAGE antioxidant (500 µl)
- Ladder (10 µl)
- Protein sample (25-50 µl)
- 4x SDS sample buffer (25-50 µl)
- SimplyBlue safe stain (25 ml)

Protocol:

1. Add 50mL 20x NuPAGE MOPS SDS running buffer to 950mL deionised water to prepare 1x SDS running buffer.
2. Obtain a NuPAGE Bis-Tris pre-cast gel, wash off with water and remove the white strip to expose a small area of the gel. If using only one gel, you must use a plastic block to ensure the upper chamber is sealed.
3. Remove the comb, slowly and carefully as not to tear the thin gel.
4. Take 200mL of the 1x SDS running buffer and add 500µl of Nupage antioxidant. Then pour this solution into the upper chamber.
5. Add 600mL of the 1x SDS running buffer into the lower chamber.
6. Load 10µl of ladder and samples in desired locations.
7. Run at 200 Volts for 50 minutes.
8. When gel has been run for the full amount of time stated, you must first empty the buffers out.
9. Take the gel and break open the casing it is in very carefully, cut the top and bottom off the gel and remove the rest of the gel into a small box.
10. Add 100mL of deionised water into the box and microwave for 1 minute on high, then put this on a Stuart see-saw rocker SSL4 for 1 minute and empty of the water. Repeat this step two more times.
11. Then add 25mL of SimplyBlue safe stain, heat in microwave for 40 seconds and put on the see-saw rocker for 5 minutes.
12. Empty the box of stain and add 100mL of deionised water to the box and put on the see-saw for 1 hour to start the destaining process.
13. The gels protein bands can then be visualised using white light.