**SOP: R004**

## Preparation of Coomassie R-250 Staining Solution for SDS-PAGE

**Materials and Reagents:** (per liter)

1. Glass beaker with a capacity > 1 liter
2. Graduated cylinders 100 ml and 500 ml
3. Stir bar
4. Magnetic stir plate
5. 1 liter glass bottle
6. Glass funnel with coarse filter paper
   or 0.45 μm Zap cap
7. Milli-Q water: 400 ml
8. Methanol: 500 ml
9. Glacial Acetic Acid: 100 ml
10. Coomassie Brilliant Blue R-250: 2.5 g (note 1)

**Protocol:**

1. _____ Measure out and pour water, methanol, and acetic acid into glass beaker containing a stir bar.

2. _____ Begin stirring on stir plate.

3. _____ Add Coomassie Brilliant Blue R-250 stain and stir for at least two hours.

4. _____ Filter staining solution using either a funnel with filter paper or a 0.45 μm Zap Cap (note 2).

5. _____ Transfer staining solution to glass bottle.

**Notes:**

1. Use only Coomassie Brilliant Blue R-250; there is also a Coomassie G-250 stain, which is used for different purposes.
2. Either method of filtration works, but using a Zap Cap with the help of a vacuum provided by the sink aspirator is much faster.

**References:**

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227: 680-685.

Coligan, J.E., et al. Current protocols in Protein Science, Volume 2, pp 10.5.10.